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2799

The soluble specific substance of a strain of Friedländer bacillus.

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By a method similar to those previously used in the case of the pneumococcus[1] there has been isolated from a strain of Friedländer bacillus designated as the "E" strain a polysaccharide with specific properties. It differs from the Friedländer carbohydrate obtained by Toenniessen[2] in giving no color with iodine, and from the material reported by Mueller, Smith, and Litarczek[3] in being nitrogen-free. $[\alpha]_D$ is + 100°, the substance, if it be a single substance, is a moderately strong acid, and on hydrolysis it yields 72 per cent of reducing sugars, among which glucose was identified as the osazone and by conversion into potassium acid saccharate. In its present state of purity this specific substance resembles qualitatively that of the Type II pneumococcus; and although there are distinct differences, it actually precipitates Type II anti-pneumococcus serum at as high a dilution as 1:2,000,000, in addition to reacting similarly with its homologous serum.

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[1] *J. Exp. Med.*, 1923, xxxviii, 73; 1924, xl, 301.

[2] *Cent. Bakt. I Orig.*, 1921, lxxxv, 225.

[3] *These Proceedings*, 1925, xxii, 373.

2800

Immunological behavior of the "E" strain of Friedländer bacillus and its soluble specific substance.

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In the preceding communication the chemical similarity of the specific soluble substance of this strain to that of the Type II pneumococcus was pointed out. These two micro-organisms, widely different in biological characters, were compared immunologically and found to correspond closely. The "E" strain and other serologically related strains of Friedländer bacillus are agglutinated by Type II anti-pneumococcus serum, and not by Type I and Type III antisera; while the Type II pneumococcus is agglutinated by "E" Friedländer antiserum, but not by the antiserum of a strain of Friedländer bacillus serologically distinct from the "E" strain. Absorption of "E" Friedländer antiserum and Type II anti-pneumococcus serum with the homologous organism removes the agglutinins for both, while absorption with the heterologous organism removes only the agglutinins for it, leaving the homologous agglutinins scarcely diminished in titer. The specific soluble substances of the two organisms under comparison react at practically as high dilutions in the opposite antisera as in the antisera to the organisms from which they were derived. Precipitin absorption parallels the agglutinin absorption. Cross-protection was equally striking, Type II anti-pneumococcus serum protecting against many thousand lethal doses of highly virulent "E" Friedländer bacillus, and the "E" antiserum protecting against Type II pneumococcus at least as well as the Type II antiserum itself. Typical protection protocols follow.

Protective Action of Antipneumococcus Serum against B. Friedländer (K).

B. Friedländer Strain "K" (Type E)	Antipneumococcus Sera		Anti-Friedländer Serum Rabbit, "E" Type 0.2 cc.	Virulence Controls
	Type I 0.2 cc.	Type II 0.2 cc.		
0.1		D 42	D 18	
0.01		D 18	S	
0.001	D 18	S	S	
0.0001	D 18	S	S	
0.00001	D 42	S	S	D 21
0.000001	D 18			D 42
0.0000001				D 19

Protective Action of Anti-Friedländer Serum against Pneumococcus Type II.

Culture Pneumococcus Type II cc.	Virulence Controls	Anti-Friedländer Serum. Rabbit immunized with Strain "E" 0.2 cc.	Antipneumococcus Serum Type II Horse 0.2 cc.
0.2		D 46	D 42
0.1		D 46	D 72
0.01		S	S
0.001		S	S
0.0001		S	S
0.00001	D 36		
0.000001	D 46		
0.0000001	D 46		

The phenomena described appear analogous to those of heterogenetic specificity among animal species.[1]

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[1] Cf. Forsman, *Biochem, Zeitsch.*, 1911, xxxvii, 78.

2801

On variants of B. pestis caviae resistant to lysis by the bacteriophage.

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In preliminary experiments last year we found that bacteriophage therapy did not influence the course of experimental mouse typhoid, produced by the feeding of *B. pestis caviæ* to mice. We were led, therefore, to inquire into the tendency of this organism to develop resistance to lysis, and to compare the virulence of such resistant strains with that of the original culture.

It was found that therapeutic administration of bacteriophage did not lead to production of resistants *in vivo* during the ten days period. Nevertheless, the mortality of animals treated during this period was the same as that among the untreated, infected controls.

Moreover, when the resistants were produced from the culture of *B. pestis caviæ in vitro*, it was found that such resistants are avirulent. When fed to or injected into mice these resistants did not recover their susceptibility to lysis, and when recovered from animals killed for this purpose they were found to remain resistant and avirulent.

These findings indicate, therefore, that the failure of bacteriophage therapy in experimental mouse typhoid is due not to production of resistants, but to failure on the part of the bacteriophage to destroy all the susceptible bacteria.

It was found that resistants isolated from the lysed cultures of *B. pestis caviæ* maintained their resistance to lysis during twenty-five passages on agar, at frequent intervals. When transferred to broth, however, one group of resistants (namely, those which yielded an agglutinated growth upon first transfer to broth) became susceptible to lysis after 5 to 7 daily passages. The other group of resistants (yielding a diffuse growth in broth) failed to become susceptible to lysis even after 120 daily passages in broth.

Simultaneously with the recovery of susceptibility, the cultures of the first group regained the degree of virulence comparable to that of the parent culture of *B. pestis caviæ*. The cultures of the second group of resistants failed to recover virulence thus far (four months after isolation). These latter cultures, apart from their lack of both virulence and susceptibility to lysis, are identical with the parent culture of *B. pestis caviæ* as indicated by their biochemical reactions, and by their antigenic properties.

The findings reported above are of interest for two reasons: In the first place they indicate that in the case of *B. pestis caviæ* there seems to exist some interdependence between the susceptibility to lysis and the virulence. Secondly they offer evidence in favor of the view that production of resistant strains is the result of selection of variants already existing in the parent culture. If resistance to lysis were to be considered the result of development in bacteria of specific resistance to the bacteriophage, it would to explain our findings, be necessary to assume (in addition to d'Herelle's hypothesis, that individual bacteria may develop immunity) that this acquired immunity is inheritable, and has been transmitted in our experiments for countless generations in the absence of bacteriophage.

2802

On the nature of inactivation of the bacteriophage by alcohol.

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Lytic filtrates are rendered inactive by the addition of an excess of alcohol.[1] If this reaction is carried out at low temperature, it becomes evident that the rate at which this inactivation proceeds is not uniform, but becomes very slow after the first few minutes. The phase of rapid initial inactivation apparently coincides with the precipitation of the filtrate by alcohol.[2] Addition of salts to the filtrate increases the rate of inactivation of the lytic principle when alcohol is added subsequently, and the higher the valency of the cation, the greater is the effect of the salt. Reduction of the salt content of the filtrate by dialysis results in the reduction of the destructive effect of alcohol upon it. It appears that in reducing the activity of the lytic agent alcohol acts not directly by virtue of its virucidal action, as assumed by d'Herelle, but indirectly by causing precipitation of the medium which, in turn, results in adsorption of the lytic principle.

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[1] d'Herelle, F., The Bacteriophage, English translation, Williams and Wilkins Co., 1922, 123.

[2] Bronfenbrenner, J., and Korb, C., PROC. SOC. EXP. BIOL. AND MED., 1923-1924, xxi, 177; *J. Exp. Med.*, 1925, xlii, 419.

2803

The inhibition of luminescence by light—Dynamics of the reaction.

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The luminescence of ctenophores is inhibited by light of a given intensity acting for a sufficient length of time. This is true for both the intact animals and for the luminescent substance obtained from them. The following experiments were made with a view to determining the dynamics of this photochemical reaction in the case of the luminescent granules of *Eucharis multicornis.*

A large specimen of *Eucharis* was shaken in a flask until all gross structures had disappeared, and the fluid remaining had the appearance of an opalescent emulsion. This was passed through a coarse filter, the resulting filtrate being a stable suspension of microscopic granules of photogenic material which, when subjected to the action of appropriate reagents, would glow with a blue-green light. In practice this was accomplished by pouring 50 cc. of the suspension into 200 cc. of tap water. The result was a bright light which lasted 5 to 7 seconds—an amount sufficient to produce deep blackening of an "extra rapid" photographic plate (Cappelli's).

In order to study the dynamics of the action of light on the luminescent granules, 300 cc. of the suspension were exposed in a broad dish to the action of a 200 cp. lamp at 50 cm. distance. At chosen intervals, 50 cc. of the suspension was removed, agitated, taken to the dark room, mixed with 200 cc. of tap water, and the photographic plate kept at a fixed distance. After the exposure the plate was numbered, then carefully covered and kept until the end of the experimental series. When the series was finished the plates were put into a large tray, and developed together for the same length of time. In similar way they were fixed, washed and dried.

The opacity, *i. e.*, blackening, of the plate is proportional to the quantity of light to which the plate was exposed. The light given off when the suspension was mixed with water was in each case a measure of the amount of unchanged photogenic material remaining in the granules. In order to obtain numbers for these quantities, the photographic plates were compared by means of a photometer. The opacity (the reciprocal of the transmission) is proportional to the intensity of the light necessary to match the constant standard lamp. The plate which records the amount of light produced by the 50 cc. of suspension which had not been subjected to illumination, is taken as equal to 100 in each series, and the opacity of the remaining plates calculated as per cents.

In all, four series were completed. Values for identical time intervals were averaged. Calculation of constants according to the formula for a monomolecular reaction yielded the following results:

Time minutes	Opacity per cent. a—x		$K = \frac{1}{t} \log_e \frac{a}{a-x}$
	Obs.	Calc.	
0	100	100	
5	77.5	83	(.050)
10	70	70	.0355
15	56	58	.0375
20	48.5	48	.0355
30	35	33.5	.035
40	20.5	23	.0395
45	17.5	19.5	.0385
55	18	13.5	.031
60	10	11	.038
			Av. = .0363
			Probable error ± .001

The photochemical reaction by which the luminescent material of *Eucharis* is destroyed therefore follows the course of a monomolecular reaction.

2804

A pernicious anaemia-like blood condition produced in monkeys with B. welchii toxin.

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In a recent article by Moench, Kahn and Torrey[1] a report was given of an analysis of the fecal flora in 33 cases of pernicious anæmia. The most striking feature revealed by these examinations was the constant presence of unusually large numbers of *B. welchii* in the stool specimens and presumably throughout the large intestine. These findings were a confirmation and extension of the earlier observations of Herter[2] and also of Simond.[3] It was suggested that the absorption of *B. welchii* toxin from the intestinal tract might conceivably be the essential etiological factor in the production of the clinical syndrome which characterizes pernicious anæmia, and that our findings justified further inquiry by experimental methods. In the present communication we wish to report in a preliminary way our observations on the effect of a potent *B. welchii* toxin on the blood of monkeys when injected intravenously.

Several strains of *B. welchii,* isolated from different cases of pernicious anæmia, were compared as regards toxin production, employing the Bull and Pritchett[4] method of production, and injecting graded amounts of the filtered toxin into the wing vein of pigeons. Our strain "Navital" proved the best suited for our purposes but it was deemed advisable to enhance its virulence by animal passages. Accordingly it was passed through six successive rabbits by intravenous inoculation, and then through four pigeons by injections into the breast muscles and recovery from the lesions on the death of the bird. The virulence of the whole culture was finally such that 0.02 cc. caused death within 18 hours. Sterile filtrates of the culture were found to be, however, only slightly more toxic than the unpassed strain.

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[1] Moench, Kahn and Torrey, *J. Infect. Dis.*, 1925, xxxvii, 161.

[2] Herter, The Common Bacterial Infections of the Intestinal Tract, New York, 1907.

[3] Simond, Monograph 5, Rockefeller Inst., 1915.

[4] Bull and Pritchett, *J. Exper. M.*, 1917, xxvi, 119.

An attempt was then made to obtain a more potent sterile toxin by a modification of the Bull and Pritchett technic, and after considerable experimentation the following method was found to yield the best results:

Medium, beef heart infusion (boiled 15 minutes), peptone (Difco) 3 per cent, casein digest fluid 10 per cent, glucose 0.2 per cent, reaction, pH 7.2. Tubed in about 20 cc. amounts in large (potato) tubes and autoclaved. Fragments of fresh pigeon breast muscle, removed aseptically, were added to each tube of medium. Before adding the tissue, the medium was heated for 15 minutes at 100° C. in the Arnold, cooled to 45°. The muscle was then added, the medium seeded and a cap of sterile vaseline applied. To prevent blowing out of this cap and hence loss of anærobiosis, the tube was sealed in the blow flame. Controls for sterility were run.

Toxin test—The culture was incubated 18 hours at 37° C., then centrifuged at low speed and finally passed through a Berkefeld N candle. The filtrate was then tested for sterility and potency. It was found that 0.3 cc. of this filtrate injected into a pigeon's breast muscle would produce massive œdema (4×6 cm. area) with characteristic blistering and terra cotta coloration, but not death, whereas 0.75 to 1 cc. was uniformly lethal within 18 hours. Within 1 to 2 hours of injection even with 0.3 cc. dosage, symptoms of profound intoxication (drooping, lethargy) became apparent. This toxin maintained its full potency for about 10 days at ice box temperature. The same dosage of the sterile medium when injected into a pigeon's breast muscle produced no reaction.

The monkeys used were the ring-tail variety *(Cebus capucinus)*. It was found that young monkeys were more susceptible to this toxin. The animals were kept on the same diet during the course of the experiment (bread soaked in milk, chopped cooked meat, bananas and occasional peanuts). After examination of the normal blood the series of toxin intravenous injections were started, using the external saphenous vein on the posterior aspect of the calf of the leg. Blood for examination was

Monkey F. Young Adult Male.

Dates	Amount Toxin Inoculated and Dates	Hemoglobin		Red Blood Cells	White Blood Cells	Differential White Cells						Anisocytosis	Poikilocytosis	Nucleated Red Cells	Punctated Basophiles	Howell-Jolly Bodies
		Per cent	Index			Polymorph.	Lymphoc. (small)	Basophiles	Large Mono.	Eosinoph.	Myelocytes					
May 29	Normal	91	1.2	3,820,000	—	25	71	0	3	0	1	—	—	—	—	—
June 1	1 cc. 5/29	70	1.13	3,120,000	—	15	81	0	3	0	1	++	—	—	—	+
June 5	2 cc. 6/1	54	1.5	1,880,000	—	5	84	0	4	3	4	+++	—	+++ 8*	+	+
June 6	3 cc. 6/5	50	1.6	1,670,000	—	20	78	0	2	0	0	+++	+	++ 3	++	+
June 8	3 cc. 6/6	45	2.5	950,000	—	30	60	0	4	0	0	++++	+++	++ 4	+++	++
June 10	None	50	1.14	2,230,000	—	50	39	0	8	0	3	+++	+++	++	+++	+++
June 12	2 cc. 6/10	52	0.93	2,730,000	5,800	61	22	0	0	3	9	++	+	+ 1	—	+++
June 15	3 cc. 6/12	56	2.0	1,440,000	1,800	51	41	0	5	0	3	++++	+++	++++ 28	—	+
June 18	1.5 cc. 6/15	65	1.19	2,940,000	5,800	48	47	0	4	1	0	+	+	+ 1	+	+
June 22	5 cc. 6/18 2 wks. old	68	1.21	2,840,000	5,000	28	65	0	3	0	3	++	— ++	—	—	++
June 26	3 cc. 6/22	52	1.3	2,000,000	2,600	41	50	0	7	1	1	++++	+++	++ 2	+	+++
June 30	7 cc. 6/26 New toxin	73	1.2	3,070,000	2,000	45	39	1	8	2	5	++	++	++ 2	— +++	+
July 6	11 cc. 6/30 Intrap.	75	0.99	3,870,000	4,100	77	5	0	8	1	9	+++	++	+ 0	+++	+ —

* Indicates number of nucleated red blood cells per 100 leucocytes counted.

obtained by puncture of the sole of the foot. The hemoglobin estimations were made with the Sahli apparatus and technic.

Three monkeys were treated, but the most striking results were obtained with two young adults, F and G. One of these, F, was given 1 to 3 cc. or more of toxin at 3 to 4 day intervals, and the other, G, 0.5 cc. doses at similar intervals. The control monkey, D, was given intravenous injections of the sterile medium. The results with monkey F are tabulated.

In all three monkeys treated with the *B. welchii* toxin changes in the blood developed which were strongly suggestive of pernicious anæmia. These included a marked drop in the erythrocyte count and in the hemoglobin percentage, rise in the index above 1.0, development of a marked condition of anisocytosis with many macrocytes and a moderate degree of poikilocytosis. These changes were noted in monkeys F and G very shortly after the first injection, and as treatments were continued became very marked. Nucleated reds also appeared, some with the morphology and staining properties of megaloblasts, together with punctated basophiles and polychromatophilia. At the stage of greatest anæmia these animals showed some desquamation of the tongue epithelium and also symptoms of nervous disturbance, irritability followed by periods of lethargy. In all the monkeys after about 20 days of treatment an immunity to the toxin began to appear and could not be broken down even with a much increased dosage as shown in the table for monkey F. For a time, however, the blood picture remained completely typical for pernicious anæmia, exhibiting every feature which is considered characteristic for that disease.

Erythrocyte count—In monkey G, which received the smaller dosage, the erythrocyte count dropped steadily from 4,150,000 to 2,060,000 during 6 days, and stayed around this level for about 20 days. With monkey F the lowest count, 950,000, was reached after 10 days. The animal became very weak and the injections were omitted for four days with a resultant rise in the count to 2,230,000, and this approximate level was maintained for 20 days, when the count began to rise in spite of increased toxin dosage.

Hemoglobin estimation—In these animals the hemoglobin reading started to drop shortly after the first inoculation. The lowest percentage attained for F was 45, and for G, 50. Both these levels were reached in a week to 10 days after the first toxin treatment and simultaneously with the lowest erythrocyte count. The hemoglobin indices were much more often above than below 1.0. In monkey F an index of 2.5 was reached at the time of the lowest erythrocyte count.

Pathological changes in erythrocytes—Anisocytosis was a very prominent feature throughout the course of the experiments. This variation in the size of the erythrocytes became noticeable within 24 hours of the first inoculation (G), and progressed to such an extreme degree that erythrocytes of normal diameter were greatly outnumbered by the abnormal types. Microcytes were at first much more numerous than macrocytes, but as inoculations were continued the macrocytes increased in number until, when the erythrocyte count was lowest, they equalled or exceeded the microcytes. The development of poikilocytosis lagged behind anisocytosis, becoming fairly marked in monkey F about 10 days after the first inoculation. In no instance was it noted in extreme degree. Nucleated reds were in evidence three days after the first toxin inoculation in monkey F. From that time to the end of the experiment they were found at nearly every examination ranging in numbers from one or two after rather prolonged search to 28 per 100 leucocytes. This last count was obtained with monkey F about two weeks after the inoculations were started, and the blood picture resembled closely that seen in pernicious anæmia during a crisis. The majority of the nucleated reds observed were normoblasts, but occasionally forms resembling young megaloblasts were noted. Fragments of extruded nuclei were encountered at times, and frequently red cells containing single light or dark blue staining dots of varying size, the so-called Howell-Jolly bodies. Punctuated basophiles were numerous at times, particularly when the anæmia was most marked.

It has been our experience with this species of monkey that the small lymphocytes are generally about as numerous as, or outnumber the polymorphonuclear neutrophiles. The toxin injections tended to increase the latter until they exceeded the former at times. Myelocytes were noted in most of the examinations and tended to increase in relative numbers toward the end of the toxin inoculations, as did also the large mononuclear lymphocytes. The leucocyte count in the normal control monkey ranged from 7,600 to 9,100, but in the toxin treated animals the count ranged from 1,460 to 5,800, with an average for monkey G of 3,000. There developed, accordingly, a definite leucopenia as a result of this treatment.

As a control on the effect of intravenous injections of the medium itself, a normal monkey was given a series of four inoculations of 5 to 8 cc. each of the sterile casein digest broth during a period of ten days. This treatment had no effect whatever on the erythrocyte count (4,520,000 to 4,750,000), the hemoglobin percentage (97 to 101), or on the morphology of the erythrocytes.

While these experiments were nearing completion an article by B. S. Cornell[5] was published in which was described the blood changes occurring in rabbits in which a chronic infection by *B. welchii* had been effected by intra-splenic inoculations. The resulting anæmia was "usually chronic and mild, but in exceptional cases, acute and profound," the resulting blood picture bearing a close resemblance to that of pernicious anæmia. The most striking feature was the anisocytosis which appeared within a few hours, at times, after the inoculation, and continued until the death of the animal. Especially interesting to us is his observation that this anisocytosis is a specific effect of *B. welchii* infection and does not follow intrasplenic injections of a variety of other bacteria including hemolytic streptococci, staphylococci and *B. coli.*

In view of the fact that we have been able to produce in monkeys all of the blood changes which are considered typical for pernicious anæmia by the injection of our *B. welchii* toxin, and Cornell obtained similar results in rabbits by instituting a chronic infection with this organism and, further, that we have demonstrated that the presence of unusual numbers of *B. welchii* is a characteristic of the fecal flora of pernicious anæmia, it would seem that this organism is deserving of further serious consideration as the etiological agent of primary importance in pernicious anæmia, as, in fact, was advocated by Herter nearly two decades ago.

In view of the above findings we believe that a test of the possible therapeutic value of *B. welchii* antitoxin in pernicious anæmia is justified.

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[5] Cornell, B. S., *J. Infect. Dis.*, 1925, xxxvi, 425.

2805

Proteins of timothy and orchard grass pollen and their reaction to vernal hay fever.

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The most widely accepted view regarding the causation of hay fever is that it is due to the pollen of certain grasses and weeds blooming in the summer and fall. Dunbar and his pupils showed convincingly that the proteins of the pollens are responsible for hay fever. There have been several attempts in the past to obtain the active constituent, but a complete, systematic chemical investigation has never been carried out. The most comprehensive study was made by Heyl[1] on ragweed. Unfortunately, however, the clinical part of his investigation was not carried out to completion.

The material used for our study consisted of the pollen of timothy and orchard grass. On account of the meager supply available, we can report only on a qualitative study.

For the exhaustive extraction, we used 5 gm. of each of the pollens, and extracted them successively with 10 per cent sodium chloride, 0.5 per cent sodium hydroxide, and 70 per cent alcohol. It is remarkable how large a percentage of the extracted nitrogen is non-protein nitrogen. From half to one-third of the total nitrogen of the pollen is not extractable with these solvents.

TABLE I.
Quantitative Analysis of Pollen.*

	Orchard grass	Timothy
	Per cent	Per cent
Nitrogen	4.51	4.00
Ash	3.98	4.31
Ether extract	3.0	3.21

* Analysis based on moisture-free material.

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[1] F. W. Heyl, *J. Am. Chem. Soc.*, 1919, xli, 670.

In the preparation study, we used about 75 gm. of the pollen, which had been previously extracted with ether to remove fatty material. It was first extracted with water, and then with 10 per cent NaCl until there was practically no nitrogen found in the extract. The residue was extracted with alcohol, and finally with 0.2 per cent sodium hydroxide. Coagulation tests made on both the water and salt extracts of the timothy pollen indicated the presence of two proteins, one coagulating at 68.8° C., and the other at 85° C. Similar extracts of the orchard grass pollen also showed the presence of two proteins, one coagulating at 65° and another at 85.2° C. We separated the lower coagulating protein by heat, and the other by ammonium sulphate at 0.65 of saturation. The latter was redissolved and dialyzed for 10 days, but the amount of precipitate formed was too small to allow a study of its properties and composition.

After all the precipitable proteins were removed from both the water and salt extracts by coagulation by heat and filtration, the combined clear filtrate was transferred to a parchment bag, and dialyzed for 10 days to eliminate the inorganic salts. The dialysate obtained was concentrated by evaporation *in vacuo*. Alcohol was added to the sirupy residue, making the final concentration 70 to 80 per cent, and the precipitated material dehydrated with alcohol followed by ether. This material obtained from the proteose fraction was easily soluble in water, and was found by the clinical tests on hay fever patients to be the most active substance of the pollen.

The residue remaining after the exhaustive extraction with water, salt and alcohol, was extracted with 0.2 per cent sodium hydroxide. By acidification of the alkaline extract with acetic acid, a protein was obtained which was found by clinical tests to have negligible importance as an exciting factor.

The clinical findings suggest that the proteose fraction is the intoxicating and primary element in all hay fever subjects sensitive to timothy pollen, and that the albumin fraction is an added toxic factor in about 50 per cent of the subjects. The importance of the glutelin fraction is negligible.

TABLE II.
Distribution of Nitrogen.

	Per cent of Total N	
	Orchard grass	Timothy
Salt soluble (10% NaCl)	17.5	9.85
Alkali soluble (0.2% NaOH)	20.8	15.4
Non-protein N	25.7	24.9
Residual N	36.0	49.8

2806

The anaphylactogenic character of horse dander and its crossed relationship to horse serum.*

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The anaphylactogenic properties of horse dander have been open to question. Friedberger[1] contends that horse dander is not anaphylactogenic. Busson and Ogata[2] and, later, Longcope, O'Brien and Perlzweig,[3] have shown that horse dander is definitely anaphylactogenic. These latter studies have appeared in the literature during the progress of our work on this same subject. We should like to state that our findings definitely show horse dander to be anaphylactogenic.

During the course of our studies, we have had occasion to work on the crossed relationship between horse dander and horse serum. Longcope[2] and his co-workers definitely state that this crossed relationship does not exist and that horse dander is anaphylactogenically specific. Contrary to these findings, we have found that there is a definite crossed anaphylactic relationship between these two substances.

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* This work is being carried on under "The Crane Fund for the Study of Anaphylaxis."

[1] Friedberger, E., and Kamio, *Ztschr. f. Immunitat.*, 1923, xxxvii, 379.

[2] Busson and Ogata, *Wien. Klin. Wchnschr.*, 1924, xxxvii, 820.

[3] Longcope, O'Brien, and Perlzweig, *J. Immunol.*, 1925, x, 599.

The difference in our results may be accounted for by the facts (1) that our method of protein extraction was somewhat different; (2) that we used horse dander as the sensitizing agent, and, (3) that we did not rely on the Dale method as a final criterion for anaphylaxis.

2807

Nasal sensitization, nasal anaphylactic shock and respiratory symptoms simulating bronchial asthma, in the guinea pig.*

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Several years ago, one of us,[1] in a clinical study of asthma in children, felt that there was strong evidence for the idea that asthma may be an "acquired" disease, and in some cases the result of sensitization through the nasal route.

Insofar as it is impossible to study the mechanism of this condition in the human being, we have applied our hypotheses to direct investigation in the guinea pig. This animal was chosen for our experiments because of the ease with which respiratory anaphylactic phenomena can be elicited.

We have found certain corroborative evidence for our results in the works of Busson and Ogata,[2] Friedberger,[3] Peragnani,[4] Sewall and Powell,[5] and Giani.[6]

Our results may be divided into three groups:

1. Guinea pigs were sensitized by exposing them to the dust of dried horse dander; subsequent intravenous injection of a protein extract of this same horse dander caused death.

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* This work is being carried on under "The Crane Fund for the Study of Anaphylaxis."

[1] Ratner, B., *Am. J. Dis. Child.*, 1922, xxiv, 346.

[2] Busson and Ogata, *Wien. Klin. Wchnschr.*, 1924, xxxvii, 820.

[3] Friedberger, E., and Kamio, *Ztschr. f. Immunitat.*, 1923, xxxvii, 379.

[4] Petragnani, *G. Policlinico, (Sez. Med.)*, 1922, xxix, 446.

[5] Sewall, H., and Powell, C., *Arch. Int. Med.*, 1915, xvi, 4, 605.

[6] Giani, E., *Giornali di Clinica Med.*, 1923, iv, 13.

2. Animals sensitized by giving them intraperitoneal injections of horse dander extract have subsequently shown grave anaphylactic phenomena and, also acute anaphylactic death when exposed to horse dander dust.

3. Animals sensitized by exposing them to horse dander subsequently exhibited marked respiratory anaphylactic phenomena when subjected to the same horse dander dust.

This latter group of animals, which at no time had received parenteral injections of the protein extract of horse dander, have presented symptoms that are comparable to the bronchial asthma of human beings. These guinea pigs have come in contact with the horse dander dust in the same manner that a human being might, for at no time was the horse dander forcibly injected into their nares.

Several animals that had previously been sensitized by an intraperitoneal injection of horse dander, died within two to four minutes after having been put in the cage containing circulating horse dander dust. There was no gross evidence of dust found in the larynx, trachea or lungs in this group of animals. This remarkable rapidity with which the sensitized animals died after coming in contact with the circulating horse dander, is directly comparable to the time elapsing after an intravenous shock injection.

We were unable to sensitize animals by feeding them dry horse dander in gelatine capsules.

We believe that the above experiments lend evidence for the probable mechanism of bronchial asthma in the human being, and further demonstrate that the nasal route may be an avenue for the production of sensitization, anaphylactic phenomena and even anaphylactic death.

2808

A new portable electrocardiograph.

HUBERT MANN.

New York City.

This instrument was developed in collaboration with L. T. Robinson, H. B. Marvin and J. K. Leibing of the General Engineering Laboratory of the General Electric Company at Schenectady, N. Y. It consists essentially of a special three stage amplifier, a special mirror galvanometer and a camera which is operated by a modified phonograph mechanism. The total weight of the instrument with batteries and all accessories in two carrying cases of rugged construction is seventy-eight pounds.

Amplification of the action current of the heart is obtained by an amplifier which resembles a radio amplifier. Two tubes of type PR-1-C and one tube of type 201-A are employed. There are special resistances so constructed as to be unaffected by temperature changes and to permit accurate standardization of the electrocardiogram. The batteries consist of a six volt storage battery and seven 22-volt dry cells.

The galvanometer consists of a small iron vane ruggedly suspended in a cell full of a clear oil of appropriate viscosity and situated in the field of a permanent magnet. To this vane is attached a small mirror. Movement of the vane is produced by means of two coils placed at right angles to the field of the permanent magnet in such a way that current passing through the coils modifies the magnetic field and causes the vane to alter its orientation. The deflection time is considerably shorter than the deflection period of the string galvanometer used in the ordinary electrocardiograph, and the viscosity of the oil in which the moving system is suspended is adjusted so as to make it dead beat. The movement of the small mirror is recorded by means of a small filament lamp the light of which is focused on the mirror by a lens and is thence reflected on to a strip of standard moving picture film. This film is moved at a constant speed by a spring motor, and is contained in a small camera which is detachable from the apparatus for removal to the dark room and subsequent photographic development.

Careful tests of the instrument and comparisons with the standard non-portable electrocardiograph have demonstrated that it gives an accurate and dependable record. Its rugged construction and reduced weight permit it to be taken to the patient's home with facility.

(The electrocardiograph was demonstrated at the meeting.)

2809

The nutritive value of the various layers of the wheat and corn kernel.

ANIELA KLEIN, CASIMIR FUNK, BENJAMIN HARROW and LOUIS PINE.

[*From the Biochemical Department, State School of Hygiene, Warsaw, Poland, the Laboratory of Biological Chemistry, College of Physicians and Surgeons, Columbia University, and the U. S. Navy Supply Depot, Brooklyn, New York.*]

Much work has been done on the relative vitamine content of the several layers in cereal grains. The peripheral layers and the germ are rich in vitamines. There is, however, not so much information available with regard to the relative content of the proteins in the various layers. The present contribution concerns itself with a chemical as well as a biological analysis of such proteins. The milling fractions of wheat and corn[1] were analyzed to determine their content of total protein, gliadin (the portion soluble in 70 per cent alcohol), globulin (the portion soluble in 5 per cent potassium sulfate solution) and glutenin (obtained by subtracting the protein representing globulin and gliadin from the total protein). The methods followed were those outlined in Methods of Analysis of the Assoc. of Official Agricultural Chemists, 1920. At the same time, the biological value of the wheat and corn fractions was determined according to Mitchell's method,[2] using rats as the experimental animals.

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[1] These samples were kindly supplied to us by the Washburn-Crosby Co.

Chemical analysis showed that among the samples of wheat (bran, middlings, red dog, first clear, second clear and patent flour), the gliadin content increases as we go from bran to patent flour; the globulin content is much higher in the bran, standard middlings and the red dog than it is in the three grades of flour; and the glutenin content diminishes as we proceed from the outer layers to what represents the patent flour. Corn shows similar results. Corn meal is richer in zein (corresponding to the gliadin of wheat) than is corn feed meal; whereas the latter is richer in glutenin than is the corn meal. Cross and Swain[3] have shown that glutenin yields more tryptophane, histidine, arginine, lysine and tyrosine, and little less cystein than does gliadin; from which, and from our own experiment, one is tempted to draw the conclusion that the peripheral layers of the cereal grains contain more of the dietetically balanced protein than does the white flour.

The animal experiments, excepting for minor details, were carried out as suggested by Mitchell (see above). The diet consisted of the milling fraction (corresponding to about 1.3 per cent nitrogen), together with 2 per cent cod liver oil, 0.5 cc. aq. sol. of rice polishing (corresponding to 1 gm. of rice polishing), 7 per cent butter, 15 per cent lard, 4 per cent salt mixture (Osborne and Mendel's), and enough starch to make a total of 100 per cent. Our results show that the fractions which contain the pericarp and the germ, and which are the ingredients found in whole grain flour, possess a higher nutritive value for rats than fractions which are composed mainly of the endosperm (patent flour). It cannot, at present, be decided whether this difference in nutritive value is due to a difference in the kind and quantity of protein, to a difference in the amount of vitamine, or to a combination of both these factors.

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[2] *J. Biol. Chem.*, 1923, lviii, 863, 905.

[3] *J. Indust. and Engin. Chem.*, 1924, xvi, 49.

2810

Suprarenal transplantation.

I. BLODINGER, H. E. KLEBANOFF and HENRY LAURENS.

[*From the Department of Physiology, Yale University, New Haven, Conn.*]

The attempt was made to obtain physiologically functionating transplants in dogs. The left gland was entirely removed, and the whole or part of it transplanted into another tissue. After an interval (5 to 80 days) the other gland was removed. No animal died after the first operation, and the immediate reaction of all was good to both. One animal lived 12 hours after the second operation; 2 lived 15 hours; 2 lived 16; 4 lived 18; 1 lived 19; 2 lived 20; 1 lived 24; 1 lived 94; 1 lived 95 hours. Thirteen, therefore, died in less than 24 hours, and two lived approximately 4 days.

Microscopic examination of the transplants showed early acquisition of blood supply. Apparently living cortical cells were present as late as 34 days after transplantation. Apparently living medullary cells were found in one case, a graft 5 days old. After 34 days no suprarenal cells could be demonstrated, the graft being completely destroyed, and partly or entirely replaced by connective tissue, phagocytic cells and a little pigment. The graft seemed to live longest in the kidney, and to be absorbed most rapidly in muscle.

2811

An unidentified base among the hydrolytic products of gelatin.

DONALD D. VAN SLYKE and WILLIAM ROBSON.

[*From the Hospital of the Rockefeller Institute for Medical Research, New York City.*]

Further preparations of the base isolated by Van Slyke and Hiller[1] have been studied. The analyses of the copper salt indicate the composition $(C_7H_9O_4N_2)_2$ Cu. The substance gives the reactions for a pyrrol group. The ratio of 1:2 for amino N: total N determined by Van Slyke and Hiller is confirmed. The behavior and analyses indicate that it may have the structure of a dihydroxy-pyrrol-alanine.

2812

Studies on the purification of antibodies. V: Nature of the pyrogenic factor in pneumococcus antibody solution.

R. OTTENBERG and F. A. STENBUCK.

[*From the Pathological Laboratory, Mount Sinai Hospital, New York City.*]

In connection with the preceding studies on purification of pneumococcus protective antibodies, it is of practical importance to know what is the reason for the occurrence of febrile reactions following the intravenous injections of the solution. The various batches of material that we prepared were tested for pyrogenic property by injecting 5 cc. amounts intravenously into rabbits and observing their temperatures for three hours.

The results which are presented on the accompanying chart indicate that the temperature rise is proportional to the amount of nitrogen present in these extracts. It will be noted that there are four results which fall outside the regular line. These show a large amount of nitrogen, but very small temperature rise. Since the nitrogen determinations were done by the regular Kjeldahl method, it is impossible to say in what form this nitrogen was. On the other hand, it will be noted that in no case in which there was a small amount of nitrogen was there a temperature rise of over 1° F. These data, therefore, while rather meagre, point to the nitrogenous nature of the pyrogenic factor. Since preceding studies by Huntoon and others indicate that the nitrogen present in these extracts comes from the bacteria and not from the antibody-bearing-serum, it seems fair to conclude that the pyrogenic factor is not inherent in the antibodies, but is derived at least in part from the sensitized bacteria.

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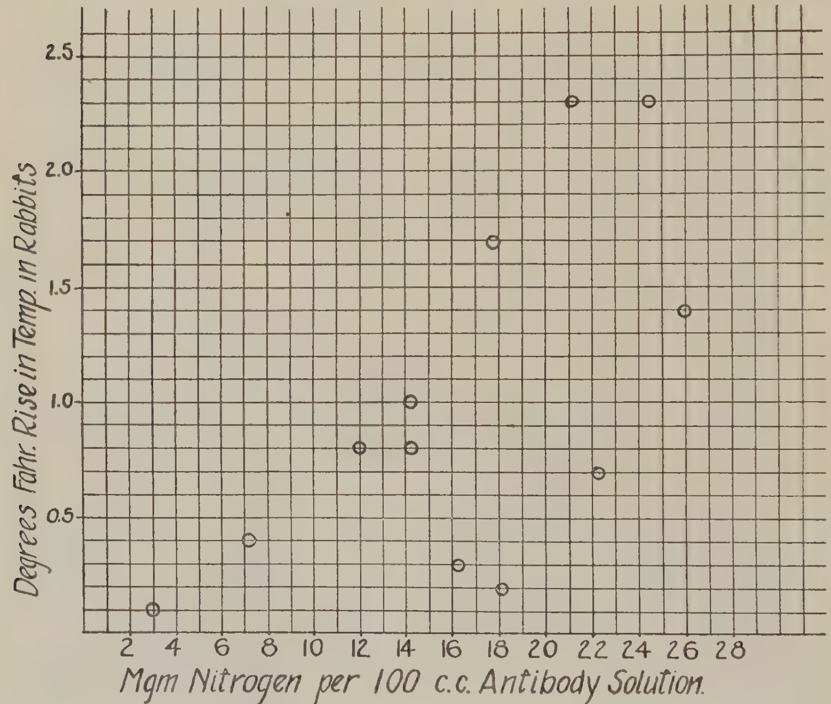
[1] Van Slyke, D. D., and Hiller, Alma, *Proc. Nat. Acad. Scie.*, 1925, vii, 185.



If the nitrogenous material derived from the antigen is the pyrogenic factor in antibody solutions, then removal of extraneous nitrogenous material by methods, such as outlined in our previous communication, may be of value in practical preparation of antibody solutions for therapeutic use.

The material and animals used in this study were generously furnished by the H. K. Mulford Co., Glenolden, Pa., through the courtesy of Dr. F. M. Huntoon.

2813

L. acidophilus in epilepsy.

NICHOLAS KOPELOFF, M. P. LONERGAN and P. BEERMAN.

[*From the Psychiatric Institute and the Manhattan State Hospital, Ward's Island, N. Y.*]

Since the gastro-intestinal tract has long been under suspicion in epilepsy, it appeared worth while to study the transformation of the intestinal flora effected by *L. acidophilus,* and any influence this effect might have upon the number of convulsions. A series of 15 epileptics were carefully observed for one month. Twelve of these then received one litre of sterile milk daily for 42 days. All 15 patients were then given one litre of *L. acidophilus* milk daily for 2 to 3 months. The methods employed have been described elsewhere.[1]

Three patients had only one convulsion per month, or less, and are therefore disregarded. Of 12 other patients studied, 4 showed a rather striking reduction in the number of convulsions. One of these, who had 122 convulsions in 23 days during the 28-day preliminary period, had an average of 5 convulsions per month for the three months during which he received *L. acidophilus* milk.

Epileptics are notoriously prone to show improvement under almost any form of treatment, but in view of the sterile milk control and another control group of epileptics who showed little benefit from kaolin administration at the same time, these results seem encouraging enough to enlarge the series. Further work is now in progress.

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[1] Kopeloff, N., *Lactobacillus Acidophilus,* Williams & Wilkins Co., Baltimore (in press).

2814

The behavior of embryonic chick gonads when transplanted to embryonic chick hosts.

B. H. WILLIER (Introduced by Frank R. Lillie).

[*From the Hull Zoölogical Laboratory, University of Chicago, Chicago, Ill.*]

Experiments were begun in the spring of 1924 for the purpose of analyzing the sex potentialities of developing chick gonads by transplanting them to the vascular chorio-allantoic membranes of host chick embryos. In these experiments the circulations of the host embryo and the sex graft are related in a manner which is essentially similar to the relations existing between twin embryos in cattle.[1] In this analysis several important problems arise. It may be possible to determine first how early in development the sex potencies of the gonads and their laterality are fixed; secondly, whether these original sex potencies may be modified or inverted by transplanting the gonadal primordia during the morphologically indifferent stage to host embryos of opposite sexes; thirdly, the behavior of sexually differentiated right and left ovaries and testes when implanted to male and female host embryos.

The method employed was essentially the same as previously reported.[2] The Wolffian body with its associated sex gland and adrenal was isolated with the aid of a binocular microscope from a donor embryo at various periods of incubation as indicated below. This isolated complex was then implanted to the chorio-allantoic membrane of a host chick embryo. In this manner the right and left gonads were implanted to male and female host embryos. The transplantations were made in all cases upon host embryos during the ninth day of incubation, and permitted to grow for an average period of nine days. The grafts were then removed and prepared for histological examination.

The sexual glands used in transplanting may be grouped into two stages of development or differentiation: (1) before sexual differentiation, that is, during the morphologically "indifferent stage"; and (2) after sexual differentiation.

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[1] Lillie, F. R., *J. Exper. Zool.*, 1917, xxiii, 371-452.

[2] Willier, B. H., *Am. J. Anat.*, 1924, xxxiii, 67-103.

I. *Grafts obtained from transplantations of gonads in a morphologically "indifferent stage"*. During this stage sex cannot be determined by the structural appearance of the gonad. This indifferent stage extends from the origin of the gonad on approximately the fourth day of incubation until the time of sex-differentiation at approximately the seventh day of incubation.[3, 4, 5] The gonads for implanting were taken from embryos incubated 4, 5, 6½ and 6¾ days. During the 4th and 5th days the gonad is in an early stage of formation, *i. e.,* the genital ridge stage; while between the 6th and 7th days the gonad is near or in the process of sexual differentiation.

After a growth period of 9 days on the chorio-allantoic membrane of the host embryo, the indifferent gonads, regardless of their ages, underwent the following differentiations. The right indifferent gonad when grown upon a male host embryo differentiates into either a testis (4 grafts) or a right ovary (3 grafts), no gonad (1 graft); when grown upon a female host embryo differentiates also into either a testis (2 grafts) or a right ovary (4 grafts). The left indifferent gonad when grown upon a male host embryo differentiates into either a testis (2 grafts) or a left ovary (1 graft), no gonad formed (1 graft); when grown upon a female host embryo it differentiates also into either a testis (2 grafts) or a left ovary (1 graft).

It thus appears from an examination of these data from 21 grafts of indifferent gonads, that the right indifferent gonad differentiates into either a testis or a *right ovary,* while the left indifferent gonad differentiates into either a testis or a *left ovary.* These differentiations take place irrespective of the sex of the host embryo.

The conclusion is therefore reached that the gonad-forming tissue is already specific and differentiated as to sex and laterality during the so-called "indifferent stage" of gonad development. Apparently, the right indifferent ovary has the potencies of a right ovary only, and the left indifferent ovary has the potencies of a left ovary only. This interpretation with respect to sex and

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[3] Firket, Jean, *Arch. de Biologie,* 1914, xxix, 201-351.

[4] Firket, Jean, *Arch. de Biologie,* 1920, xxx, 393-516.

[5] Swift, C. H., *Am. J. Anat.,* 1915, xviii, 441-470.

laterality is possible since the right and left grafted ovaries show the same differences in size and structure as normal right and left ovaries (the right and left grafted testes like the normals cannot be distinguished histologically). Thus it is apparent that the sexual potencies are determined in the indifferent gonad before morphological evidences for sexual differentiation appear. In other words, the morphologically indifferent gonad is not physiologically indifferent.

How early in the development of the embryo the specific organization of the gonad with respect to sex and laterality appears is an interesting question. Such an organization is already established in the indifferent gonad at the approximate time of its origin in a 4-day embryo (this statement is supported so far by only three grafts). As yet this is the earliest stage that I have attempted to transplant. Whether or not this specific organization of the gonad is established before the fourth day may possibly be analyzed by transplanting the right and left primordia of the Wolffian bodies so as to include the immediately surrounding peritoneum from chicks incubated less than four days.

The primordia of the right and left gonads apparently have the capacity for independent growth and differentiation, for they differentiate according to their specific organizations, and are not modified by the sex of the host embryo. This is demonstrated (1) by the differentiation of the right indifferent gonad, whether implanted to a male or a female host embryo, into either a testis or a *right ovary,* and (2) by the differentiation of the left indifferent gonad, whether grown upon a male or a female host embryo, into either a testis or a *left ovary.* These results prove that hormonic sex-differentiating factors are either absent in the host, or, if present, they are ineffective in the modification of the sex graft in the developing chick.

The indifferent gonads, therefore, seem, except for dependence for nourishment from the blood stream of the host embryo, independent of the host surroundings in the attainment of specific differentiation. Whether or not the differentiation of the indifferent gonad is independent of the associated adrenal and Wolffian body of the graft is an open question. This may be tested by implanting an indifferent gonad which has been completely isolated from its associated organs (this ought to be possible on at least the fifth day of incubation).

II. *Grafts obtained from transplantations of sexual glands after the occurrence of sexual differentiation.* From the end of the seventh day of incubation the sex of the gonads may be distinguished with certainty by differences in size and particularly form.[6] The ovaries and testes for implanting were obtained from embryos incubated 7, 9, 10, 11, 12 and 13 days respectively. After a growth period of 9 days on the chorio-allantoic membrane of the host embryo, the graft was removed and examined histologically. To date 46 such grafts have been examined.

Right ovarian grafts. The right ovary when grown upon either a male (4 grafts) or a female (10 grafts) host embryo is unmodified in its essential structures. It is histologically similar to the right ovary of a normal chick of late embryonic stages. So far no cortex (second set of sexual cords) has been distinguished. In the majority of grafts the gonad consists of a distinct ovarian medulla. This medulla is composed of the usual structural components of an ovarian medulla, such as the epithelial or medullary cords (often in the form of small epithelial groups or as isolated cells in some grafts) and a connective tissue stroma.

There are considerable variations in the form, the size and the texture of the implanted right ovary. These variations, however, could not be associated with the sex of the host embryo, but are apparently conditioned by the exigencies of the experimental procedure of grafting.

Left ovarian grafts. The left ovary retains its essential structures when grown upon either a male (6 grafts) or a female (3 grafts) host. The engrafted left ovary shows a distinct cortex and medulla, which are histologically similar to these regions in a normal left ovary of the later stages of embryonic life. The cortex consists of groups of epithelial cells including primordial germ cells. The medulla has the same components as described for the right ovary. The implanted left ovary shows variations in form and size, in the thickness of the cortex, and particularly in the texture of the medullary region. These variations of the medulla are of the same sort as occur in the engrafted right ovary, and are apparently similar to the "Phénomènes dégénératifs des cordons médullaires" of the right and left ovaries described by

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[6] Swift, C. H., *Am. J. Anat.*, 1916, xx, 375-410.

Firket.[3, 4] These variations of both cortex and medulla cannot be correlated, so far as at present ascertained, with the sex of the host embryos but are more probably associated with the isolation and implantation of the graft.

Right and left testicular grafts. The right testis was grown upon either male (7 grafts) or female (5 grafts) hosts; the left testis upon either male (3 grafts) or female (5 grafts) hosts. An examination of these testicular grafts shows that in all cases the essential testicular structures such as sexual cords (potential seminiferous tubules) containing supporting epithelial cells and primordial sex cells, intercordial tissue, and a more or less well-developed *tunica albuginea,* are present. These structures are similar to those of normal testes of the late embryonic stages. The engrafted testes vary in form, size and topographical relations to the Wolffian body. It is quite evident that these variations from the normal, as in the grafts of right and left ovaries, are not casually related to the sex of the host embryo, but rather are due to the operative conditions.

Finally the question is raised as to whether the engrafted gonads modify the development of the sex organs and ducts of the host embryo. These host embryos occasionally show such atypical features as (1) variations in the size and form of the sex glands, (2) the persistence of a complete right Müllerian duct in a female, and (3) the presence of both right and left Müllerian ducts in a male embryo. These atypical structures, however, are not specifically related to the sex of the engrafted gonad, and they appear in control embryos on which grafts of various endocrine glands from adult chickens have been growing.

2815

A thyroid-adrenal interrelationship.

R. L. ZWEMER (Introduced by W. W. Swingle).

[*From the Osborn Zoological Laboratory, Yale University, New Haven, Conn.*]

Evidence has accumulated during the last few years that the cortical portion of the suprarenal complex of mammals is essential for life. This has been clearly demonstrated by the work of Houssay and Lewis,[1] Wislocki and Crowe,[2] and the present writer.[3] In a series of experiments performed upon forty cats, the writer was able to show clearly the great importance of cortical tissue for the maintenance of life. The following points were brought out: (1) Cats, from which the adrenals have been removed (in two stages 7 days apart), die within three days. (2) Unilaterally operated animals remain perfectly normal. (3) Total extirpation of one adrenal, and removal of the medullary portion of the other (after 7 days), leaving only the cortex, does not induce symptoms, the animals remain normal. (4) Removal of the remaining cortical tissue after an interval of 21 days to three months, causes death within three days. (5) Histological examination of the removed cortex showed no medullary tissue to be present in the majority of cases. (6) Transplantation of pieces of the cortex into animals with both adrenals removed, prolonged the life of the animals. During the course of the experiments mentioned, an interesting functional interrelationship between the suprarenal cortex and the thyroid gland was established. It was observed that thyroidectomized cats survived removal of the suprarenal glands much longer than animals with intact thyroid apparatus. In experiments on twenty animals this point was thoroughly investigated.

The thyroid glands and the left adrenal were removed at one operation, ten days later the right adrenal was extirpated. Following thyroid removal the parathyroids were dissected free from their investing membranes in a medium of sterile Ringer's solution and blood serum[4] and transplanted into the neck muscles, to prevent tetany.

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[1] Houssay, B. H., and Lewis, J. T., *Am. J. Physiol.*, 1923, lxiv.

[2] Wislocki, G. B., and Crowe, S. J., Johns Hopkins Hosp. Bull., 1924, xxxv.

[3] Zwemer, R. L., *Anat. Record*, 1924, xxix.

The life of animals from which the thyroids and adrenals had been removed was considerably prolonged over that of similarly operated cats in which the thyroids were left undisturbed. The average survival period of cats with the adrenals removed (in two stages 7 days apart) is three days, whereas the average survival of thyro-adrenalectomized cats is three times as long, or about nine days. One of these animals survived 22 days.

In order to determine the effects of feeding thyroid to normal animals, four adult cats were each fed four grains of dessicated thyroid (Parke, Davis & Co.) per day. Two animals received the thyroid for two weeks, two for one week. All showed an increased appetite and a decrease in weight, but no unfavorable symptoms of any kind were observed. The next step was to test the effect of feeding thyroid to adrenalectomized animals. The thyroid glands and left adrenal were removed, and, during the ten day interval between the removal of the left and right adrenals, the animals received four grains of dessicated thyroid per day. At the end of ten days the remaining adrenal was removed. Death resulted within 12 to 23 hours. This short survival period cannot be attributed to faulty technique, because in all cases the cats recovered from the anesthetic and behaved normally for some hours after the operation. Autopsies showed the wound areas to be clean, and the gross appearance of the viscera to be normal.

One animal developed typical symptoms of adrenal insufficiency and died 9 days after the first operation, although still retaining one adrenal gland. This case was complicated, however, by the occurrence of transient tetany on the third and fourth day after thyroid removal.

These experiments demonstrate a functional interrelation between the thyroid and adrenal glands, in that the life of animals deprived of their adrenals, can be greatly prolonged or shortened by absence or excess of the thyroid hormone.

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[4] Swingle, W. W., and Nicholas, J. S., *Am. J. Anat.*, 1925, xxxvi.

2816

A method for the quantitative determination of small amounts of quinin and quinidin with bromin water.

SOMA WEISS and ROBERT A HATCHER.

[*From the Department of Pharmacology, Cornell University Medical College, New York City.*]

We undertook to learn whether quinin could be determined quantitatively in extracts of animal tissues by means of the so-called thalleioquin color reaction, but it soon became apparent that the intensity of the color varies with too many factors to permit of its use for this purpose. It was observed, however, that the color of the bromin water disappeared sharply on the addition of a slight excess of quinin, and the present method depends upon the fact that when quinin is added to bromin they combine in definite proportions with the loss of the color of the bromin in reflected light.

Hart[1] attempted unsuccessfully to estimate quinin quantitatively by means of the thalleioquin reaction. He found, however, that four atoms of bromin are absorbed by the quinin, two of them loosely. He says, "It follows, then, that quinine may be determined from its bromine absorption for five minutes in the manner stated: 1 cc. N/10 Br. = 0.0081 g. quinine." This method has not been employed hitherto so far as we know.

Nine solutions of quinin, varying widely in concentration and containing from 0.5 to 2.0 milligrams of quinin base, were prepared by one of us (H) and examined by the other (W), who did not know the strength of the solution in any case. The determinations were made with an average error of about five per cent.

The necessary reagents are prepared in the following manner: Bromin water: Place a convenient volume of bromin in a bottle with a tightly fitting glass stopper, add water, and shake until saturation occurs with an excess of bromin in the bottom of the bottle. It keeps indefinitely.

Diluted bromin water: Dilute a convenient volume of bromin water with nine volumes of water immediately before using. This is placed in a bottle having a tightly fitting glass stopper and protected from light.

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[1] Hart, W. B., *Soc. Chem. Ind. J.*, 1921, lx, 72.

Standard quinin solution: Dissolve 120.1 milligrammes of quinin hydrochlorid (U. S. Ph.) in enough distilled water to make 100 cc. One cc. of this solution corresponds to 1 milligramme of anhydrous quinin base.

Standard quinidin solution: Dissolve 120.6 milligrammes of quinidin sulphate (U. S. Ph.) in enough distilled water to make 100 cc. One cc. of this solution corresponds to 1 milligramme of anhydrous quinidin base.

Method of procedure: Place 0.5 to 1.0 cc. of diluted bromin water, accurately measured, in a test tube of about 5.0 cc. capacity and of about 10 millimeters diameter; add the standard quinin solution until the color of the bromin is discharged. One milligramme of quinin (1. cc. of the standard solution) corresponds to about 0.85 cc. of the diluted bromin water, hence one must add about 1.2 cc. of the standard quinin solution to 1.0 cc. of diluted bromin water.

The disappearance of the color of bromin is observed best by reflected light with the tube held against a white background. A yellowish tint may still be observed by looking down through the depths of the solution. This must be disregarded. All glass used must be free from alkali and the test tubes must be free from a yellow color. The average of at least 3 readings is taken, and these must not vary more than 3 per cent.

Determination of quinin in the extract: The extract or substance to be tested should be made into a solution having as nearly as possible the same concentration of quinin as the standard, but in any event, not less than 1 milligramme of the base in 5 cc. of solution. When an extract from tissue is to be tested, it is convenient to add a few drops of alcohol to the extract with 0.25 cc. N/10 sulphuric acid for each milligramme of extract and warm. When solution is complete, it is diluted so that 1 cc. contains about 1 milligramme of the extract. Tests showed that traces of alcohol and a slight excess of sulphuric acid are without influence on the accuracy of the determination.

The solution of the extract is tested with the same amount of diluted bromin water and exactly as described for the standard quinin solution. When the preliminary test shows that 1 milligramme of the extract contains less than 0.2 milligrammes of quinin, the extract must be repurified, if possible.

Calculations of quinin in the unknown: The number of cc. of the standard solution of quinin required to decolorize the diluted bromin water is divided by that required of the solution of the extract. The quotient is the amount of quinin in milligrammes (or fraction of a milligramme) in 1 cc. of the solution.

When numerous solutions are to be examined, the standard quinin solution should be tested after three or four solutions have been titrated. The reason for this precaution is that the bromin evaporates, and after a series of tests requiring an hour or more a slight loss in the concentration of the diluted bromin water may occur.

When diluted bromin water is allowed to stand at rest for several hours, the upper layer becomes colorless while the lower layer changes much less, or not perceptibly, when the container is tightly stoppered. The pipette used for measuring the diluted bromin water should be of such capacity that a residuum of bromin water remains in the pipette after the required amount is discharged. The balance in the pipette is thrown away. The loss of bromin can be avoided or minimized by the use of petrolatum on the stopper, but this is inconvenient owing to its adherence to the pipette.

Quinidin is estimated in exactly the same manner, but the standard is titrated against a known amount of quinidin when quinidin is to be determined quantitatively. In each of two tests of one specimen of quinidin, it required 8 per cent more diluted bromin water than an equal weight of the specimen of quinin used.

2817

The experimental production of a relative immunity to the cerebral manifestations of lead poisoning.

CARL VERNON WELLER.

[*From the Department of Pathology, University of Michigan, Ann Arbor, Michigan.*]

When guinea pigs are poisoned with lead in the form of commercial white lead administered by mouth in capsules, with a daily dosage of about 300 mg. per kilo of animal, convulsions result on about the fourth day. These convulsions are highly characteristic, being epileptiform in character and exhibiting both clonic and tonic phases. Such animals may die in the first, or in subsequent convulsions, or, if the administration of lead be stopped, they may recover.

If a resting period of sufficient length be allowed after the cessation of convulsions, the survivors will be found to have acquired a partial immunity in respect to the cerebral manifestations of lead poisoning, so that they may be given a larger number of doses of the same size as those first used without producing convulsions. If this process be repeated, the amount of lead which can be administered without producing convulsions may be greatly increased. A limited number of animals thus prepared have reached a point at which four times the usual convulsion-producing dosage has been given without convulsions resulting. During each of the longer periods of administration the usual loss of weight, cachexia and muscular weakness develop, indicating that whatever immunity is produced is against a local action of lead and not against its general toxic effect. There has been noted a marked individual variation in the ability of animals to develop this relative immunity, and in certain instances death has occurred unexpectedly in animals well along in the course of lead administration.

The experimental evidence thus obtained is in accord with the belief of some of the older writers upon lead encephalopathy, that the manifestations of this condition are more apt to be shown by those relatively new to a particular lead hazard than by those who have been long exposed to lead. Clinically, there are found numerous exceptions to this rule, although our experimental results indicate that it is founded upon a scientific basis.

2818

The experimental production of lead gangrene in guinea pigs.

CARL VERNON WELLER.

[*From the Department of Pathology, University of Michigan, Ann Arbor, Michigan.*]

Kazda[1] has reported three clinical cases of gangrene of the feet in relatively young typesetters. These he believes to be the first clinical observations of lead gangrene. Similar experimental results have been obtained by us in guinea pigs which have received large amounts of white lead by mouth. In this animal there can be produced a dry gangrene of the ears. The extreme margin of the pinna becomes slightly shrunken with a hyperemic zone proximal to the border. The margin to a varying depth becomes necrotic, drys down and is finally cast off, leaving an irregular margin which is somewhat thickened. The condition is usually bilaterally symmetrical. This result has been obtained only in those animals which have received an unusually large amount of lead, such as is made possible by the method described in the preceding communication. That it has not been observed hitherto is apparently due to the fact that it has been impossible to administer a sufficient amount of lead without killing the animal through lead convulsions. The induction of a partial immunity makes this possible.

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[1] Kazda, F., *Wien. klin. Wchnschr.*, 1923, xxxvi, 694.

2819

Skin tests on hay fever cases with (chemically) different fractions from ragweed pollen.

A. H. W. CAULFEILD.

[*From the Connaught Laboratories of the University of Toronto, and the Toronto D. S. C. R. Chest Clinic, Toronto, Canada.*]

During the course of some experiments on anaphylaxis in guinea pigs with solutions made from ragweed pollen, it was found desirable to work with extracts of more definite or purer chemical composition. This was accomplished through the co-operation of Dr. George Eadie (Department of Bio-Chemistry, University of Toronto). With certain of the solutions thus obtained, Miss Cecile Cohen has been able to induce both active and passive anaphylaxis. Further, it would appear, that, though injections with one fraction will induce an anaphylactic condition to that particular fraction, the animal may not be sensitive to other fractions, even though these other fractions can act as satisfactory sensitizing antigens in varying degrees.

Reports upon both the chemical and immunological aspects of the experiments are in the course of preparation for publication.

Shortly after the earlier results were obtained with guinea pigs it seemed desirable to see what information could be obtained by using the different fractions for skin tests with cases of hay fever known to be sensitive to ragweed. The method used was a skin scratch of about 2 to 3 mm. in length upon which a droplet of NaOH solution was placed and the dried fraction rubbed in with a blunt glass applicator.

The present report is to show that ragweed cases, as judged by the results of the skin tests to (chemically) different fractions of ragweed, exhibit a distinct and individual variation.

While as many as 16 different preparations have been used for the tests, 4 fractions have seemed of more importance than the rest. These are (1) a mixture of albumin and proteose; (2) proteose; (3) glutenin; and (4) a 95 per cent alcoholic extract. To afford a measure of control either dried pollen, acetone precipitate bodies (prepared as advocated by Bauman, Chudnoff and

Mackenzie[1]) or a glycerine and salt solution, diluted 1-4, has been used as well.

The results of the tests in 10 of 50 cases tested are given in Table 1. These were obtained by taking in rotation the first and second examples in which the maximum skin reaction was obtained respectively to the albumin and proteose fraction, to proteose, to glutenin, and to the alcoholic extract. In estimating a maximum reaction the so-called controls were not considered. Two more, showing a different combination of results, were added. The 10 examples serve to show that each case will have its own relative degree of positiveness to one or more of the fractions. Tests with any of the so-called controls, namely, the acetone precipitate bodies, whole pollen, or glycerine-salt extract give no indication of this.

As controls 2 normal individuals and 3 grass positive cases of hay fever were tested and found to be negative. In several cases the tests were repeated without relative or material variation. In 2, the albumin and proteose fraction was omitted.

The following summary gives approximately the percentage results for each fraction:

1. Cases in which the albumin and proteose fraction gave the maximum test were 39 in number (78 per cent). Of these 25 (50 per cent) gave no—or at the most a comparatively slight—reaction to proteose, and can in consequence be considered predominantly or exclusively sensitive to ragweed albumin.
2. Cases, in which the proteose fraction gave a marked test approximately equal to any except the control substances, were 17 (34 per cent) in number. Of these, 3 seemed to have an additional, though less marked, sensitization to albumin.
3. With glutenin the tests were negative in 21 cases (40 per cent). The reaction was slight or moderate (comparatively) in 18, and marked in 11 (22 per cent).
4. With the alcoholic extract the tests were negative in 26 cases (52 per cent). The reaction was slight or moderate (comparatively) in 14, and marked in 10 (20 per cent).

From the few cases of hay fever, positive to timothy grass, which have been tested with similar fractions of timothy pollen varying results have been obtained as with the ragweed cases.

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[1] Bauman, Chudnoff, and Mackenzie, PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 226.

Summary: Tests with chemically different fractions of ragweed pollen have been shown to give varying (or no) degrees of positiveness in cases of hay fever: used as antigens in experimental anaphylaxis, there would seem to be comparatively slight evidence of cross sensitization.

Such results suggest that clinically more satisfactory desensitization might be achieved if the preparations of ragweed used were made to correlate with the results of the tests in the individual case.

TABLE 1.
Results of skin tests in selected ragweed cases.
(Figures given are millimeter measurements of wheal.)

	Ragweed fractions										
	Alcoholic Ext.	8x8	7x5	20x15	neg.	15x8	4	6	8	neg.	neg.
	Glutenin	neg.	16x6	neg.	10x8	neg.	?	neg.	neg.	8x6	neg.
	Alb. and Proteose	18x15	11x11	12x10	10x7	18x15	8	18x12	18x15	25x25	7x6
	Proteose	5	10x8	10x8	neg.	neg.	10	4x4	5x5	15x8	neg.
Controls	Glycerin Salt Ext.	8x8	5x6	—	—	—	10	7x6	8x8	—	—
	Aceton ppt. bodies	16x11	—	10x10	14x14	6x6	—	—	16x11	—	—
	Pollen	11x10	8x6	7x7	5x5	7x7	8	7x7	11x10	7	3

2820

Influence of nutritive condition on initial fall in blood sugar after insulin.

MAX TIITSO (Introduced by J. J. R. MacLeod).

[*From the Laboratory of the Insulin Committee, University of Toronto, Toronto, Canada.*]

In order to investigate whether prolonged starvation has any influence on the initial fall in blood sugar after subcutaneous injection of insulin, rabbits were starved for 1 or 2 weeks, and the results obtained were compared with rabbits well fed with carrots.

All the rabbits were injected with the same dose of insulin, namely 3 units per 2 kilo body weight. The blood was taken every ten minutes after injection for an hour. The blood sugar was determined by the Shaffer-Hartmann method. After the last sample of blood had been taken, the animal was instantly killed by stunning, and the liver and muscles of the leg removed for determination of glycogen. The glycogen was determined by Pflüger's method.

I. *Rabbits fed with carrots.*

3 hours previous to experiment, animals starved in order to decrease any absorption from the intestine.

Norm.	10 mins.	20 mins.	30 mins.	40 mins.	50 mins.	60 mins.
0.1365%	0.140%	0.097%	0.075%	0.072%	0.074%	0.062%

Average glycogen in the liver 5.5%, and in the muscle 0.24%.

II. *Rabbits starved for* 1 *week.*

Average for 7 animals.

Norm.	10 mins.	20 mins.	30 mins.	40 mins.	50 mins.	60 mins.
0.130%	0.135%	0.121%	0.107%	0.096%	0.082%	0.076%

Average glycogen in the liver 0.83%, and in the muscles 0.125%.

III. *Rabbits starved for* 2 *weeks.*

Average for 3 animals.

Norm.	10 mins.	20 mins.	30 mins.	40 mins.	50 mins.	60 mins.
0.141%	0.140%	0.118%	0.100%	0.104%	0.101%	0.093%

Average glycogen in the liver 0.24%, and in the muscles 0.021%.

An examination of the curves shows that there is in some cases a slight rise in blood sugar 10 minutes after the injection of insulin. This rise is probably not caused by insulin, but by emotional disturbance. It is commonly seen in rabbits that are not injected with insulin, and usually amounts to between 5 and 14 mg. In the next 20 minutes the sugar fell much more rapidly in the fed than in the starved animals. The decrease in the fed animals was 61.5 mg., and in the starved 23 and 41 mg. per 100 cc. of blood.

The starved animals were apparently more resistant to insulin an hour after injection than the fed ones, and this resistance increased with the duration of starvation.

In fed animals the blood sugar fell in an hour by 74.5 mg.; whereas in starved ones, only by 54 mg., or 48 mg. per cent.

Abderhaldvn and Wertheimer[1] have found that rats fed with carbohydrate-rich diet are much more susceptible to insulin than are rats fed with carbohydrate-free diet. The fall in the blood sugar was greater in the carbohydrate rats than in rats fed with carbohydrate-free diet.

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[1] Pflüger's Arch., 1924, 203 and 205.

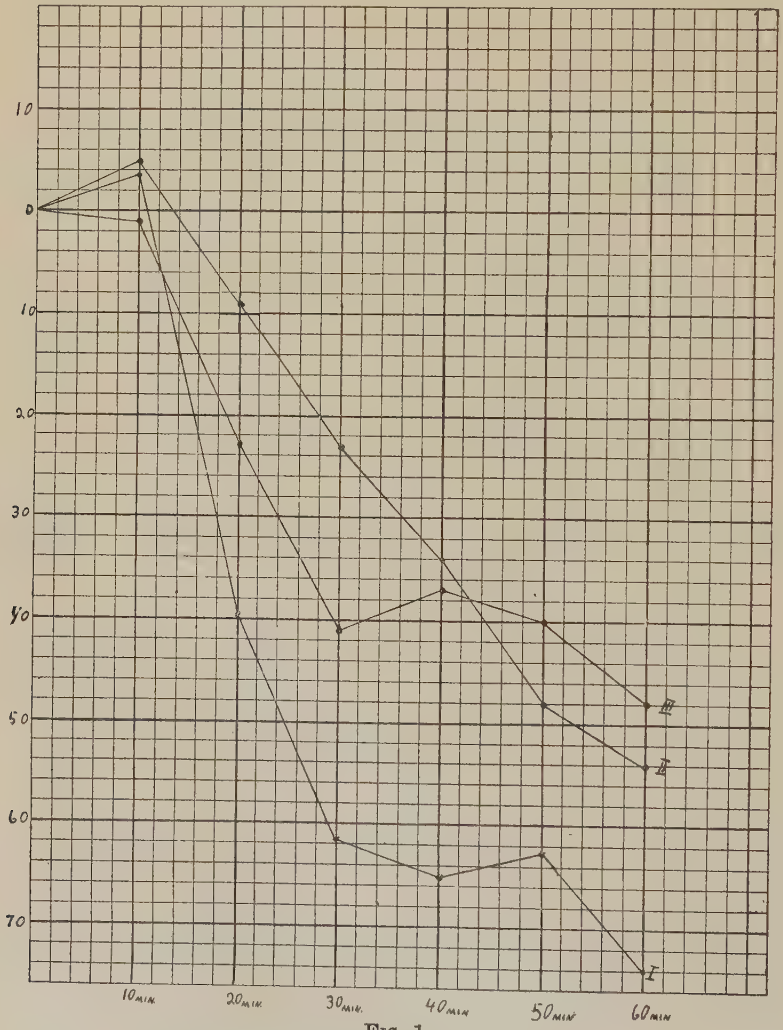

FIG. 1.

Curves of results recorded in tables. The ordinates indicate fall in blood sugar in mg. per 100 cc. blood. The abscissæ time in 10 minute intervals. The curves show the average blood sugar: I, in 7 rabbits fed with carrots; II, in 7 rabbits from which food was withheld for one week; III, in 3 rabbits similarly treated for two weeks. All results are brought to the same value to start with.

It seems, therefore, that ingestion of carbohydrates makes the organism in some way more susceptible to insulin; whereas absence of carbohydrate obtained by starvation, or administration of carbohydrate-free diet, decreases the hypoglycæmic effect of insulin.

2821

Fate of non-native varieties of colon-aerogenes in intestinal tracts of young chicks.

MILDRED WINCHESTER (Introduced by Emerson Megrail).

[*From the Department of Hygiene and Bacteriology, Medical School, Western Reserve University, Cleveland, Ohio.*]

Starting with sixteen 24-hour-old chicks, the feces were examined for the presence of citrate and non-citrate-using members of the colon-aerogenes group of bacteria for a period of one week. No growths of colon organisms having the ability to utilize citrate as a sole source of carbon were obtained, but small numbers of citrate-using *B. aerogenes* were found in the feces of nearly all of the chicks.

Eight of these chicks were fed "non-fecal" (citrate-using) *B. coli* at the rate of two agar slants per chick per day, the organisms being washed off into the milk at the morning and night feedings. All bacteria used were growths from single cell isolations, the coli being soil organisms of the Koser type,[1] and one culture was furnished by Dr. Koser.

During the first three weeks the experiment was conducted under sterile conditions in so far as the colon-aerogenes group was concerned; all food was thoroughly pasteurized, all dishes were sterilized at each feeding, and the chicks were kept in sterile cages containing sterile sand and a layer of sterile shavings. During the latter part of the experiment, sterilization was discontinued since the number of colon-like organisms present in the food, etc., before sterilization was too small to make any difference in the enormous amounts fed to the chicks.

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[1] Koser, S. A., *J. Bact.*, 1924, ix, 59.

Specimens of feces in the first period were obtained aseptically. A suspension of a portion of the feces from each chick was made in sterile broth, and from this, eosin-methylene-blue-crystal violet-agar[2] plates were streaked. (This medium gave a very sharp differentiation between colon and aerogenes types, but no satisfactory test as between colon types using or not using citrate.) From the colon colonies which appeared on these plates, ten were picked at random and transferred to citrate medium. In the latter part of the experiment no precautions were taken in regard to sterilization with no apparent change in results.

During continuous feeding of these large amounts of citrate-using *B. coli* over a period of three months, they appeared in the feces to the extent of 26 per cent of the colonies examined. Several times during this period they disappeared, sometimes for four or five days. Their disappearance occurred more frequently during the third month of the experiment than at first. Upon cessation of feeding, however, they disappeared *entirely,* always within three days of the time the feeding was discontinued.

Five control chicks, kept under normal conditions and fed on unsterilized food without addition of citrate-using bacteria, were run at the same time, but at no time during the experiment were any colon organisms found using citrate.

Three additional chicks were used in an attempt to supplant *B. coli* with *B. aerogenes* in the intestinal tract by consistent feeding of these organisms. The feeding of a non-native *B. aerogenes* produced no relative increase in the numbers of these organisms. The feeding of a native type—found normally in the feces of the chicks in small numbers—resulted in raising the number of organisms to 40 per cent of all colonies examined. These, like the citrate-using *B. coli,* disappeared almost immediately when the feeding was discontinued.

Thus, although it is apparently possible, by persistent feeding, to implant temporarily these citrate-using bacteria in the intestinal tracts of young chicks, it is merely a transitory thing, since the organisms disappear entirely as soon as the feeding is discontinued, and even disappear intermittently while the feeding is in progress. It is evident, therefore, that these non-native types of bacteria meet with some adverse conditions in the intestine of the chick. These conditions would appear to be due to one of three factors:

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[2] Skinner, C. E., and Murray, T. J., *J. Infect. Dis.*, 1924, xxxiv, 585.

(1) An *inhibition* of the organisms owing to an unfavorable environment or to antagonistic action of other organisms;

(2) The *destruction* of the bacteria due to (a) the influence of an abnormally high temperature on these soil organisms; (b) the chemical activities in the body of the chick; (c) the chemical activities of other organisms.

(3) The *transformation* of the citrate-using bacteria into the non-citrate using type in the intestinal tract of the chick.

Of these factors, the temporary inhibition of the organisms appears to be the least probable; a month after inoculation of a known mixture of the two types into citrate medium, both are found viable when transferred to eosin-methylene-blue plates, thus eliminating any shorter time factor; yet when similar transfers are made from negative citrate tubes, no growths of citrate users develop. Investigation of these separate points in now in progress in the hope of obtaining more definite information concerning the fate of the citrate-using bacteria in the intestinal tracts of young chicks.

2822

Chronic ulcerations in the dog's stomach produced by x-ray.

JOHN A. WOLFER (Introduced by Lester R. Dragstedt).

[*From the Laboratories of Surgery and Physiology, Northwestern University Medical School, Chicago, Ill.*]

Progress in the study of gastric ulcer has been decidedly delayed by the fact that it has been impossible to produce, experimentally, chronic ulcerative lesions in the stomach of the lower animals, notably in the dog. Innumerable attempts have been made in the past twenty or more years, and, with one possible exception, they have all been failures. In certain instances, Dragstedt was able to produce a chronic ulcer by injecting 4 per cent silver nitrate solution beneath the mucosa, and then stitching in and out through this area, using non-absorbable material.

In about 60 per cent of cases, a chronic lesion was produced, but the procedure was by no means dependable, and added the element of a foreign body imbedded in the tissues.

In October, 1923, I began an experimental study of gastric ulcer. Accepting the suggestion given me by Dragstedt, I began a series of experiments to determine whether the exposure of the mucous membrane of the dog's stomach to X-ray would produce a destructive lesion that would be slow in healing. Our opinion was that, since X-ray burns of the skin were slow in healing, often resisting for months all forms of treatment, if such a lesion could be produced in the wall of the dog's stomach, it might be considered a pathological and clinical counterpart of chronic ulcer in the human. This may be questioned, at least until microscopic evidence will indicate whether these experimentally produced ulcers are histologically similar to human chronic ulcers.

After a number of failures due to insufficient exposure, it was finally discovered that an ulceration which runs a definite chronic course, can be produced in the dog's stomach by the X-ray.

The following technique and protocols are submitted as a preliminary report:

The dog's stomach is delivered as far as possible through an abdominal incision. An incision over the pre-pyloric antrum is made through the anterior wall, parallel to the long axis of the stomach. Bleeding vessels are ligated if necessary. The posterior wall of the stomach is everted through the incision of the anterior wall, and four sutures are placed in the posterior wall so as to represent the four angles of a square. These sutures are threaded through a lead plate which has a foramen about 3 cm. in diameter. When these sutures are tied, an area of the posterior wall is fixed in the foramen of the lead plate, and is then exposed to the rays. Occasionally it will be necessary to place a few extra sutures, fixing the posterior to the anterior wall, to prevent leakage of gastric contents. An exposure of 150 milliampere minutes, 140 KWV, unfiltered, at a distance of nine inches is given. The fixation sutures are then cut, and the wall of the stomach is closed by two layers of sutures.

In a relatively small number of dogs which have been examined, this method has not failed in producing a chronic ulcer.

PROTOCOLS.

Dog 7. Exposure 3-20-24. On 5-28-24, a definite chronic ulcer, 14 mm. long, 6 mm. wide and 2 mm. deep, present. A scar on the posterior serous coat. On 2-26-25, ulcer still present, practically same size, edges more indurated. Animal still alive.

Dog 8. Exposure 5-11-24. On 3-12-25, a definite chronic ulcer present. An area about 2½ cm. in diameter, indurated and roughened. In the center of this area, a fissure 8 mm. in length, 3 mm. in width, and about 3 mm. in depth. No scar on the posterior serous coat. Animal still alive.

Dog 9. Exposure 6-13-24. On 3-5-25, a chronic pear-shaped ulcer, 1 cm. in diameter and 2 mm. deep, present. Floor of the ulcer granulating. Dog still alive.

Dog 15. Exposure 2-24-25. On 5-15-25, an ulcer 4 mm. long, 2 mm. wide, oval in shape, punched out appearance. Dirty grey floor, edges indurated, surrounded by a hyperæmic area. Dog still alive.

Dog 12. Exposure 3-17-25. On 5-19-25, died of a generalized peritonitis due to a gastric perforation. Perforation about 6 mm. in diameter, edges thickened. Microscopic examination of the margin of the perforation shows but little sign of inflammatory reaction. Most of the necrotic tissue digested away.

Proper anesthesia was used in all cases.

2823

Analogous action of insulin and epinephrin on the liver.

ERNST F. MÜLLER and WILLIAM F. PETERSEN.

[*From the Department of Preventive Medicine, College of Medicine, University of Illinois, Chicago, Ill.*]

Insulin and epinephrin are frequently considered as antagonistic because hypoglykemia and hyperglykemia follow after the respective administration of these substances.

Since we know the mechanism of insulin action,[1] the alleged antagonism of insulin and epinephrin gained interest for us. We have previously demonstrated that insulin has a two-fold action depending upon whether or not insulin is absorbed at the site of injection.[2]

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[1] E. F. Müller, H. E. Wiener, and R. vE. Wiener, Proc. Soc. Exp. Biol. and Med., 1925, xxii, 375.

From a tissue depôt (an intradermal injection) insulin acts purely by nerve stimulation, increasing the glycogenetic function of the liver. This glycogenesis finds its expression in a consequent lowering of the blood sugar. Insulin absorbed from its tissue depôt, or injected intravenously does not bring about this glycogenetic action.

Insulin injected intravenously or absorbed from a subcutaneous injection becomes effective after making contact with the tissues of the body. This process is one that takes a certain period of time, and the effect will not be noted for 30 to 40 minutes after injection, while the nerve action is immediate. Insulin injected subcutaneously or intravenously, for comparison with epinephrin action, will enable us to compare only the hormone action of insulin, *i. e.,* its glycolytic action, with the effect of epinephrin. Such an antagonism is quite improbable. The antagonistic process would consist of building up sugar.

Pertinent experiments of MacLeod and his associates[3] have been made as follows: Epinephrin was given to animals one hour after insulin injection, with a resulting equalization of both insulin and adrenalin effect on the blood sugar. We do not believe that this observation justifies the assumption of an antagonistic effect of epinephrin and insulin.

In a series of animals we have first determined the relative dosage of epinephrin and insulin which would produce a corresponding percentage augmentation or reduction, respectively, of blood sugar. The result showed that an average .05 mg. of epinephrin and 0.5 units of insulin per kilogram body weight increased or diminished the blood sugar approximately 50 per cent in two hours time. Simultaneously injected (subcutaneously, either separately or mixed) insulin and epinephrin do not show an antagonistic action in the majority of cases. In more than 60 per cent the result is an increased insulin action, a typical lowering of the blood sugar, in spite of the presence of enough epinephrin to theoretically balance the insulin effect.

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[2] E. F. Müller and W. F. Petersen, same, in print.

[3] J. J. MacLeod, *Physiol. Reviews,* 1924, iv, 21.

If we give epinephrin 20 minutes before the subcutaneous injection of insulin, a decrease of the blood sugar follows. This decrease exceeds the effect of a single corresponding insulin injection in the same animal in the majority of cases; in some instances a decrease of 60 per cent is reached in two hours.

Since we know the two-fold action of insulin, the explanation of these observations is simple. Epinephrin acts by increasing vasocontraction and diminishing endothelial permeability; it postpones and diminishes the absorption of insulin injected simultaneously or later. Consequently the insulin, excluded from the circulation, does not come into contact with the tissues, and glycolysis does not take place; but from the tissue depôt the nerve action of the insulin becomes manifest. Glycogenesis is stimulated, and continues active as long as insulin is not absorbed. So epinephrin, through its vaso-constricting effect, postpones insulin absorption, makes possible its glycogenetic nerve effect, and eliminates for this period of time its hormone action. The glycogenetic effect of insulin is presumably much stronger than the glucose mobilization initiated by an epinephrin deposit in the tissues because an increase in blood sugar is usually absent.

Examining our results in connection with those of MacLeod, who injected epinephrin during the period of most intensive insulin action, the following might be mentioned before discussing the underlying explanation of these findings. In a previous communication[4] we have shown that subcutaneous injection of insulin is followed without exception by an increase of blood sugar, while intradermal injections result in an immediate blood sugar decrease. We have also demonstrated that intradermal injections act glycogenetically, while insulin present in the circulation (as after subcutaneous and intravenous injections) mobilizes glucose. Thus glycogen is depleted in the liver.

It may be of interest to mention that probably insulin, *per se,* when present in the blood stream, acts in mobilizing sugar. Were this glucose mobilization to depend only on a replacement basis (sugar vacuum of the tissues) it would not be demonstrable in the short interval following injection, nor would it reach the high levels which we have obtained during the period immediately after injection (50 per cent or more). During this time the *in vitro* effect of insulin is not apparent.

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[4] E. F. Müller and W. F. Petersen, *J. Am. Med. Assn.,* in print.

Insulin present in the blood stream almost instantly mobilizes glucose, and in this way increases the blood sugar level. The mechanism of this action is still unknown. Insulin may act by direct contact or by a sympathetic effect on the liver.

The result of the mobilization of glucose from the liver, cannot be determined longer than about 20 to 30 minutes after the injection, because at that time the hormone effect becomes apparent and conceals the further mobilization of glucose, which is instantly acted upon by the combined tissue-insulin effect. With these considerations in mind we believe that MacLeod's, as well as our own experiments, can be interpreted as follows:

(1) Epinephrin given before or simultaneously with insulin delays the absorption of insulin; the nerve effect of the insulin depôt initiates glycogenesis in the liver, and decrease in blood sugar follows.

(2) Epinephrin given an hour after insulin injection (when insulin has been completely absorbed and has reached its maximum effectiveness) mobilizes glucose from the liver, and thereby increases the already existing glucose mobilization. The glycogen store of the liver may thereby be practically exhausted, but the blood sugar level is maintained by the sufficiently rapid mobilization of glucose from the liver.

This interpretation, that insulin and epinephrin have an analogous influence upon the liver, may be confirmed in various ways. We have already succeeded in demonstrating that the effect of the injection of insulin (subcutaneous and intravenous) acts in mobilizing glucose.

It is also known that animals respond to drugs with a varying degree of intensity. For instance, rabbits which responded only slightly to insulin administration, react very markedly to epinephrin. One rabbit, for example, reacted with a decrease of only 22 per cent of its previous blood sugar content (uniform dosage of 0.5 units per kilogram body weight); while another showed a decrease of 65 per cent in the same interval. It was of interest to find that the former showed an increase of 125 per cent of its sugar level after the administration of epinephrin, while the latter responded with a 40 per cent increase (uniform dosage of 0.5 mg. per kilogram body weight). More examples of such individual differences were observed, not all of the same striking degree.

The individual difference is explicable on the basis already suggested of an analogous action of the epinephrin and insulin. Both epinephrin and insulin act by depleting the liver of glycogen. If the liver response is individually intensive, much glucose will be mobilized and will be found in the circulation after epinephrin administration. This same animal will also release considerable amounts of glucose during insulin action, thus almost completely replacing the metabolized sugar, and the sum total of the insulin effect as observed in the blood sugar curve will be relatively small.

If, on the other hand, the liver of another individual releases but small amounts of glucose to an irritation, the demonstrable epinephrin reaction will be small, and only a part of the metabolized sugar (after insulin injection) will be replaced, so that a distinct sugar deficiency becomes apparent in the blood.

The observations indicate that, while the hormone action of insulin is always in direct proportion to the amount of insulin present in the tissues, the liver response depends very largely on the individual reactivity of the particular animal, seemingly a fixed characteristic of that organism.

The individuality of the response is of importance for the study of the nerve action of both epinephrin and insulin, while it is, of course, of lesser interest for the hormone effect of insulin which must be considered an independent process.

The individuality may also be of importance for the understanding of the described analogy of insulin and epinephrin.

An analogous glycogenolytic effect of insulin and epinephrin is demonstrable if sufficient amounts of insulin and epinephrin are present in the circulation.

2824

Chronic nephropathy induced by prolonged administration of an alcoholic beverage. Recovery experiments.*

WM. de B. Mac NIDER.

[*From the Laboratory of Pharmacology, the University of North Carolina, Chapel Hill, North Carolina.*

In a recent publication[1] observations were recorded concerning the toxic effect (on kidneys of normal and of naturally nephropathic dogs) of an alcoholic beverage produced by fermenting with yeast a mixture of either corn meal and sugar or meal and molasses. The distillate from such a fermented mixture had an ethyl alcoholic content which varied 28 to 42 per cent. When normal dogs were given 10 cc. per kilogram of such a distillate, they developed a loss of muscular co-ordination and a staggering gait which was followed by a period of complete muscular relaxation and sleep. The distillate was given to the animals by stomach tube once a day over periods which varied from six weeks to three months. In such animals the functional evidence of renal injury consisted in an increase in the twenty-four hour output of urine, later followed by a reduction in urine formation below the amount established as normal for the animal before the use of the distillate. The urine from all of the animals showed albumin which varied from a trace to 4.8 gm. per liter. Casts of the hyaline or finely granular variety were only occasionally observed. During such intoxication the elimination of phenolsulphonephthalein was but slightly reduced. There was no retention of blood urea or nonprotein nitrogen. The reserve alkali of the blood remained unaffected.

The twelve dogs used in this study were killed at periods of six weeks or three months from the commencement of the use of the alcoholic distillate. The histological study of the kidneys of the animals showed an acute injury which was very largely confined to the glomeruli. The capillary loops were engorged with blood, and the endothelial cells of the loops showed a marked deposition of stainable lipoid material. Such material was also found in

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* Aided by a grant from the Ella Sachs Plotz Foundation.

[1] Mac Nider, Wm. de B., *J. Pharmacol. and Exp. Therap.*, 1925, xxvi, 97.

the cells of the loops of Henle. In this latter location this material is normal for the dog kidney. In the convoluted tubule cells fine, dust-like particles of lipoid material could rarely be demonstrated.

The animals in this initial series of experiments have shown a lack of functional disturbance of the kidney, except a marked albuminuria with occasional hyaline and finely granular casts, and a variation in the output of the watery element of the urine. In the previous publication the suggestion was made, on the basis of functional and histological findings, that the altered output and composition of the urine might be explained on the assumption of an increase in the permeability of the glomerular vessels.

RECOVERY EXPERIMENTS.

Fourteen normal dogs were given 10 cc. per kilogram of an alcoholic distillate obtained from the fermentation by yeast of a mixture of corn meal and sugar. In addition to this fluid, the animals were given by stomach tube 250 cc. of water twice a day. The distillate was administered for ninety consecutive days. Its use was then stopped, and the animals were further studied at intervals of four days for eight months in order to obtain observations associated with the changes in renal repair during this period of recovery. At the end of the eight month period the animals were killed, and the kidneys studied histologically.

During the period of ninety days when the animals were receiving the alcoholic distillate, the changes in the urine were in general similar to those outlined in the preceding group of experiments. The detailed account of the two experiments which follow are characteristic for the animals of this series during the period when the distillate was being used as well as for the period of recovery when the use of the distillate had been discontinued. The animal of Experiment 2 had a normal daily output of 427 cc. of urine which was free from albumin, glucose and ketone bodies. The two hour elimination of phenolsulphonephthalein was 79 per cent. Blood urea was 12 mg. and non-protein nitrogen 27 mg. per 100 cc. of blood. The alkali reserve of the blood was 8.1. On the fifth day of the use of the distillate, the urine had increased to 789 cc. It contained 3.8 gm. of albumin per liter and an occasional hyaline cast. The elimination of phenolsulphonephthalein was reduced to 65 per cent. There was no retention of blood urea or nonprotein nitrogen. The reserve alkali of the blood was unaffected. On the sixtieth day of the use of the distillate, the urine contained 6.2 gm. of albumin per liter. There was apparently no increase in the number of casts. The elimination of the dye was 67 per cent. The blood urea, nonprotein nitrogen and reserve alkali of the blood remained unchanged. The output of urine on three consecutive days was 741 cc., 589 cc., and 702 cc.

Two months after the use of the alcoholic distillate was discontinued a study of this animal gave the following findings: The animal had a twenty-four hour output of urine of 784 cc. The urine contained 1.9 gm. of albumin per liter. The elimination of phenolsulphonephthalein was 65 per cent. There was no change in the blood findings other than a slight reduction in the blood urea of from 12 to 10 mg. per 100 cc. of blood.

At the termination of the experiment after a period of eight months had been allowed for the recovery, the output of urine on four successive days varied from a minimum output of 431 cc. to a maximum output of 914 cc. The urine contained only a trace of albumin, and very rarely a hyaline cast. The phthalein elimination was 68 per cent, as opposed to the normal elimination for this animal of 79 per cent. The blood urea and nonprotein nitrogen determinations during the course of the experiment showed but slight variations from the normal. There was no change in the reserve alkali of the blood.

The animal of Experiment 10 has shown in general the same type of response as the animal of Experiment 2. Prior to the use of the distillate, the animal had an average daily output of urine of 508 cc. which was free from albumin, casts, glucose and ketone bodies. The elimination of phenolsulphonephthalein in a two-hour period was 80 per cent. The blood urea was 18 mg. and nonprotein nitrogen 30 mg. per 100 cc. of blood. The reserve alkali of the blood was 8.0. On the ninetieth and final day of the use of the distillate the animal had an output of urine of 530 cc. The urine contained 2.4 gm. of albumin per liter, and showed occasional hyaline and finely granular casts. The elimination of phenolsulphonephthalein was 70 per cent, the blood urea determination was 14 mg. and the nonprotein nitrogen 32 mg. The reserve alkali reading was 8.05. At the termination of the experiment, eight months after the discontinuance of the distillate, the average daily output of urine was 714 cc. The urine contained 0.8 gm. of albumin per liter. The elimination of phenolsulphonepthalein was 73 per cent, as contrasted with the normal elimination of 80 per cent. The blood contained 14 mg. of blood urea, and 36 mg. of nonprotein nitrogen. The reserve alkali of the blood was 8.0.

The histological study of the kidneys of these twelve animals which were given a period of eight months in which to recover from the effect of the alcoholic distillate, shows a chronic type of injury confined to the glomeruli. The injury is uniform in its distribution, and in this respect differs markedly from such injuries developing as a result of bacterial invasion of the glomeruli. The lobulation of the capillary tufts has disappeared. The capillary loops appear matted together. Many of the loops have become obliterated by the proliferation of large spindle shaped cells. Mitotic figures have been found in six glomeruli in which this type of cell proliferation was taking place. These cells appear different from the ordinary fibroblast. They are larger, and the cytoplasm of the cell is clearer. In capillary loops in which the cell proliferation is sufficiently marked there is an absence of blood cells. In other capillary loops of the glomeruli, where the process of cell hyperplasia has not been so marked, the loops are distended with blood. There has been no demonstrable thickening of the capillary wall. In a few glomeruli the most advanced evidence of injury is shown by areas of hyaline degeneration. The capsular epithelium has failed to show evidence of hyperplasia. The capsule of the glomeruli and the pericapsular area has not shown connective tissue formation. The chronic injury at this stage of its formation is certainly a pure intracapillary reaction. The tubular epithelium has shown no evidence of injury.

CONCLUSIONS.

1. The chronic renal injury in dogs following the use of the alcoholic distillate when sufficient time is allowed for the development of processes of repair, consists in a type of glomerulonephropathy, characterized by the obliteration of the capillary loops by a large type of connective tissue forming cell. The capsule of the glomerulus does not participate in the reaction. The epithelial cells in the various portions of the tubule appear normal.

2. Following the glomerular injury which may have advanced to the stage of hyaline degeneration of the capillary loops, there occurs only slight evidence of an impairment of renal function. Urine from such kidneys constantly contains albumin and occasional hyaline and finely granular casts. The twenty-four hour output of urine when studied on successive days is variable. There is no constant decrease in the output. In four of the animals the output of urine has been markedly in excess of the intake of fluid. Such animals have shown a reduction in body weight. With the development of this type of glomerular injury there has been no other marked evidence of an impairment of renal function. The elimination of phenolsulphonephthalein has not been below 60 per cent in any of the animals. There has been no retention of blood urea or nonprotein nitrogen. The reserve alkali of the blood has been unaffected.

3. The kidney may be the seat of a diffuse and advanced type of chronic glomerular injury with only two indications of a functional disturbance: a marked variation in the twenty-four hour output of urine, and a urine which contains albumin and an occasional cast.

Missouri Branch.

Washington University Medical School, May 20, 1925.

2825

Effect of ion combinations on protoplasm, amoeboid movement, tissue formation in experimental amoebocyte tissue.

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In former investigations we have shown that the consistency and state of contraction of the protoplasm determines various functions and transformations in amœbocytes and in the experimental amœbocyte-tissue, such as: (1) The character of the amœboid movements and pseudopods. The greater the consistency, the more rounded are the cells, the sharper are the pseudopods, the slower is the movement of the granuloplasm. At certain stages the movement of the granuloplasm is entirely or almost entirely prevented, while the ectoplasm of the pseudopods may still actively move. Then the cell *in toto* may move in a snail-like manner, the whole cell being pulled along by the extension and contraction of the pseudopods. However, snail movement may also be produced under other conditions. The other extreme is represented by the circus movement and paripassu movement. In the former, the pseudopod has the shape of a drop or balloon, and moves along the periphery of the cell; the granuloplasm pours into it like a liquid mass. Subsequently the cell as a whole rounds off, and the same cycle begins again after a period of latency. In the latter form of movement, the protoplasm is so soft that the pseudopod is rounded off, and the granuloplasm moves into the pseudopod as soon as it develops, so that the pseudopod is from the beginning filled with granuloplasm.

(2) The consistency of the protoplasm influences the extension of the cell. The greater the consistency, the less is the cell liable to extend in contact with solid surfaces; conversely, the softer the cell, the more liable it is to extend. Associated with

the extension are usually solution processes in the cell, in particular, solution of the cell granules. However, other factors in addition to extension, influence such solution processes; thus it may occur that in extended cells the granules remain preserved for some time at least; and, conversely, in contracted cells the granules may be lost. The chemical constitution of the fluid surrounding the cells largely determines these conditions.

(3) The agglutination of the cells depends upon the consistency of the protoplasm. A very great consistency diminishes the agglutination of the amœbocytes to one another as well as to the glass by making the surface of the cells hard. Stickiness of a gelatinous material depends upon a limited taking up of fluid on the part of the gelatinous material.

(4) Agglutination, extensive migration of the cells, and the character of the pseudopods and cell outlines, together with solution of granules determine the production from amœbocytes of the tissue-like structures, most often resembling mesenchymatous tissue, but also, under certain conditions, epithelial and other kinds of tissues. The consistency of the protoplasm which is largely influenced by the environment, and also by differences in the cells of various individuals, determines the character of the tissue-like structure resulting from these transformations.

(5) The character of the degenerative changes taking place in amœbocyte tissue depends largely upon the consistency of the cells. In very consistent tissue hyalinization of the contracted cells may occur. With intermediate stages of consistency, vacuolization and the transformation of the tissue into fibrillar structures of various kinds may take place; or individual cells may disintegrate into a mass of cell granules. If the consistency is very low, the whole tissue may disintegrate into an opaque, structureless mass.

The consistency and state of contraction of the protoplasm depends: (1) On inner factors which vary in the case of different individuals. Amœbocytes derived from *Limuli* which are in a bad condition of health, tend especially to be very soft and to show little resistance. (2) On physical condition of the environment. A low temperature tends to increase the consistency and state of contraction, while, within a certain range, a higher temperature favors softness and extension. (3) On the chemical constitution of the fluid which surrounds the tissue.

If we consider the action of various ions on the consistency of the protoplasm of amœbocytes, and on the correlated phenomena mentioned above, each ion has a characteristic effect. If we take serum as a standard, sodium tends to cause contraction of the cell and to lead to hyalinization. Potassium in small amounts causes a softening, leading to circus movements, especially in combination with hypotonicity; in stronger concentration it causes fixation and death. It tends to preserve the granules. It alters the normal plasticity of the cell, either in the direction of too great a liquidity and mobility, or in the direction of too great a stiffness, depending upon the concentration of the potassium and associated factors, such as osmotic pressure, temperature, simultaneous action of other ions, and character of the individual amœbocyte tissue. The effect of calcium and rubidium is intermediate between that of sodium and potassium. Ammonium does not permit normal movements of the cells. It leads to the formation of multiple drop-pseudopodia and mulberry cells. Ca leads to solution processes, hyalinization and cytolosis and complete disintegration of the amœbocytes. The anions we can arrange in the following order: (1) NO_3, (2) Cl, (3) SO_4. NO_3 tends to cause softening; SO_4 hardening of the cells; and Cl exerts an intermediate effect. H-ions in the form of HCl or other acids tend to increase the consistency of the amœbocytes. OH-ions tend to preserve the cells, temporarily, if used in a higher concentration. Gradually the solution processes predominate. If weaker concentrations are used the softening affects predominate from the beginning. In former investigations we influenced the consistency of the cells and the associated changes through combinations of various ions. Thus through combination of NaCl solutions and acid we could increase the consistency of the cells, improve the preservation and increase in certain cases the outgrowth of the tissue.

During the summer of 1923, with Miss Elizabeth Gilman, we determined the action of various ion combinations on the consistency of amœbocytes and the associated phenomena mentioned above. We repeated and extended these experiments during the summer of 1924 with Mr. H. Beerman and Miss Ida P. Genther. We found that the effect of various combinations of ions on the consistency, amœboid movement, agglutination, tissue formation and degeneration of amœbocytes can be predicted on the basis of the action of the individual ions, provided we take into consideration also the concentration of the various ions, the osmotic pressure, the temperature of the solutions, and the character of the tissue used. Various ions may mutually antagonize or intensify their separate actions according to the character of the combinations. This rule applies in cases in which a kation and an anion are united in the same salt, as well as in cases in which several salts are combined in the solution. Thus SO_4 counteracts the softening effect of low concentration of K more effectively than does Cl; while NO_3 under certain conditions intensifies softening and toxic effects. Therefore KNO_3 will neutralize the hardening effects of Na_2SO_4 more completely than KCl. A combination of NaCl and KCl can be as effective as a combination of Na_2SO_4 and KNO_3 The results also depend on the character of the tissue. A naturally soft tissue is comparable to a more resistant harder tissue to which a small amount of KCl or a still smaller amount of KNO_3 has been added. A harder, more resistant tissue resembles a softer tissue to which an adequate amount of acid has been added. If, on the basis of such and other similar considerations, the consistency of the cells can be predicted, we can therefrom also predict the action of the various ion combinations on amœboid movement, character of pseudopods, agglutination, extension of the cells, tissue formation, rapidity of outgrowth and secondary degeneration in the amœbocyte tissue.

2826

Decolorization by acids and alkalis of amoebocytes and of filter paper stained by neutral red.

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We have previously found[1] that amœbocytes of *Limulus* stained with neutral red are almost instantaneously decolorized by N/1000 HCl in isotonic NaCl solution. Addition of weak alkali (N/1000 NaOH) causes the granules to stain again, provided sufficient stain is available in the surrounding solution. At that time we had referred already to experiments in which we compared the action of acid and alkali on amœbocytes, and, on filter paper, both stained with neutral red. We now wish to report more definitely on the comparison between the extraction by acid and alkali of neutral red and eosin from filter paper and from amœbocytes stained with these substances.

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[1] Leo Loeb and Elizabeth Gilman, *Am. J. Physiol.*, 1924, lxii, 526; Leo Loeb and K. C. Blanchard, *Biol. Bulletin*, 1924, xlvii, 284.

In the case of both amœbocytes and filter paper stained by neutral red, acid is more active in extracting the stain than either alkali or water or a neutral salt solution. If we use a series of watery solutions of HCl in strengths varying between N/100 and N/5000 HCl, the neutral red is extracted the more readily the stronger the acid. If the corresponding solutions of HCl are made up in 3 per cent and 10 per cent NaCl instead of in H_2O, there is still some indication that the stronger solution of acid extracts more stain than the weaker solution; but on the whole the differences between the effect of the stronger and weaker solutions, if present at all, are much less marked than in the case where the pure watery solution of HCl are used. This diminution in the differences between different strengths of acid in NaCl solutions as compared to watery solutions is due (1) to a weakening of the extracting power of the stronger HCl solution through the admixture of NaCl to the acid; this weakening effect is much greater in 10 per cent NaCl than in 3 per cent NaCl. (2) This is due to the fact that the weaker NaCl solutions (1 per cent and 3 per cent NaCl) increase the extracting power of weak (N/5000) HCl solution as compared to the extracting power of a watery solution of the same strength of acid. This is in accordance with the fact that H_2O extracts practically no neutral red, while neutral 1 per cent to 10 per cent NaCl solutions extract a very small amount of neutral red from the filter paper. Stronger solutions of alkali (N/100 to N/500 NaOH) extract less neutral red than acid solutions of the same strength. Weaker alkali solutions (N/2000 to N/5000 NaOH) extract on the whole still less than the stronger solutions; but the difference between the various strengths of alkali is much less marked than that between corresponding extractions of acid solutions.

From filter paper stained with eosin, alkali extracts more stain than acid. This holds good especially if we compare the stronger (N/100 to N/500) concentrations. Weaker solutions (N/2000 to N/5000) of acid extract more stain than the stronger solutions of acid; and the difference between acid and alkali almost disappears in the case of weaker concentrations. In watery solutions of acid and alkali the extraction is somewhat better than if the acid and alkali are used in the 3 per cent to 10 per cent NaCl solutions. In pure H_2O and in neutral 1 per cent to 10 per cent NaCl solutions only a trace of eosin is extracted.

Summary.—Acid and alkali behave oppositely as far as their extracting power towards an acid and an alkaline dye is concerned. Addition of NaCl inhibits the extracting power of stronger concentrations of acid and in certain cases also that of alkali towards neutral red. Neutral NaCl solutions on the other hand extract neutral red better than water. How far in these effects the dissociation depressing action of NaCl or other actions of NaCl are concerned is uncertain. These experiments demonstrate that there exists a certain similarity between the action of acid and alkali on amœbocytes on the one hand, and on filter paper on the other hand; but this simlarity is incomplete. It therefore appears probable that the conditions determining the staining of cell granules by neutral red differ in some essential respects from those determining the staining of filter paper.

2827

The effect of insulin upon the metabolism of certain bacteria.

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The striking pharmacological effect of administering insulin to a diabetic person suggests that a similar response might possibly be induced in cultures of bacteria that do not utilize glucose, provided, of course, that insulin alone, and no supplementary factor, is responsible for the phenomenon.

Four organisms that do not utilize glucose (or any other carbohydrate) were tested with this possibility in view. They were *Bacillus alcaligenes, Micrococcus catarrhalis,* a vibrio isolated from a diseased alimentary tract, a previously undescribed coccus. None of these microbes induces measurable chemical changes in glucose, so far as available methods indicate.

Plain glucose and glucose-insulin broths were prepared, and inoculated with parallel amounts of the respective organisms. Incubation was practiced for 6, 24, 48 and 72 hours respectively. At these several intervals the various media were examined for evidence of fermentation, that is, for change in hydrogen ion concentration, titratable acidity, and for gas formation. None occurred. The reaction changes were uniformly toward the alkaline side, indicating the utilization of the protein constituents for energy. In other words, insulin failed to induce these non-glucose fermenting organisms to utilize the sugar.

Shortly after these observations were completed, Noyes and Estill[1] reported experiments indicating that insulin increased the rate of fermentation of glucose, when it was added to cultures of *Bacillus bulgaricus* and *Bacillus acidophilus.* Their procedure was to add to a milk-broth-glucose solution some insulin, and, with suitable controls without insulin, observe the titratable acidity at suitable intervals. They reported that a 20 to 25 per cent increase in acidity was detectable in cultures of *Bacillus bulgaricus,* considerably less in corresponding cultures of *Bacillus acidophilus,* where insulin was used.

These experiments were repeated, following the technique of Noyes and Estill, but with negative results. Both the coagulation time, and the rate and intensity of acid production proceeded at a uniform rate in cultures with and without insulin. It is possible that the organisms used in the two laboratories were not the same. All that can be said is that the cultures of *Bacillus bulgaricus* and *Bacillus acidophilus* tried here were typical in all respects.

It is important for several obvious reasons to know whether any bacteria can be influenced in their glucose metabolism by insulin. Therefore a third series of tests were set up, using a variety of microbes of varying fermentation reactions, and of widely different proteolytic capacities. They included: the dysentery bacilli, typhoid, the paratyphoid bacilli, the Morgan bacillus, coli, proteus, cholera and *Streptococcus hemolyticus, Straphylococcus aureus,* and types of pneumococci. The cultures were examined under parallel conditions in glucose and glucose insulin broths, at the same intervals as were those in the first series. It should be stated that 20 units of insulin were used for every 50 cc. of broth culture. A Lilly preparation, which gave satisfactory clinical reactions, was employed. In every instance the results were negative.

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[1] *Proc. Nat. Acad. Sc.,* 1924, x, 415.

These observations seem to indicate that insulin added to glucose media has no apparent effect either upon the rate or the intensity with which several types of bacteria utilize glucose for energy, as measured by changes in titratable acidity and by changes in hydrogen ion concentration.

2828

Identification of two types of mononuclear phagocytes in the peripheral blood of rabbits.

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Mallory[1] in 1898 named the mononuclear phagocyte of typhoid fever lesions endothelial leukocytes. Since that time cumulative evidence has given much support to the view that the mononuclear tissue and blood phagocytes arise from endothelium. Aschoff and Kiyono[2] by means of vital dyes traced these free cells to the reticulo-endothelium. Sabin[3] in the chick embryo saw endothelial cells detach themselves from the walls of veins and become free in the lumen of the vessel; and this author,[4] after marking the vascular endothelium by the intravenous injection of carbon in suspension, observed the carbon-laden endothelial cells to undergo mitosis and to separate and assume the same morphology as the free cells.

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[1] Mallory, F. B., *J. Exp. Med.*, 1898, iii, 611.

[2] Aschoff, L., and Kiyono, K., *Folia. Hæmatol.*, 1913, xv, 383.

[3] Sabin, F. R., Contributions to Embryology, 1920, iii, Carnegie Inst. Wash. Pub. No. 272, p. 213.

[4] McJunkin, F. A., *Am. J. Anat.*, 1919, xxv, 27.

Recently Sabin, Doan and Cunningham[5] gave the first noteworthy evidence that these phagocytes are not of a single type, by demonstrating in peritoneal exudates with supravital stains a cell with the neutral red granules grouped in the hof of the nucleus in "rosette" form, in distinction to a second type with scattered neutral red granules. The author[6] repeated their experiments and found, in addition to the two types described by them, a third or hyaline type with little affinity for neutral red. Examination by the author of the lymph nodes, spleen and liver, fresh and in paraffin sections with the neutral red mordanted *in situ,* gave evidence which appears to be conclusive that the rosette type of cell first described by Sabin and co-workers, Cunningham and Doan, arises from the reticulo-endothelium of the lymph nodes, while the hyaline type, since it so frequently contains carbon after the intravenous injection of ink, was regarded as a cell derived from the blood vascular endothelium. The cell derived from the lymph vessel endothelium, as well as the one derived from the blood vessel endothelium, was found under certain conditions to present neutral red granules scattered irregularly in the cytoplasm; but the diffuse granulation was found especially in the large young cells and in the actively phagocytic ones of lymph node origin.

The author at an earlier date adopted the term of endothelial leukocyte, which was proposed by Mallory, for the mononuclear phagocytes. Separation of the group into the lymph-endotheliocytes of lymph vascular origin and the hem-endotheliocytes of blood vascular origin now appears to be warranted by sufficient data. The presence of the former in the normal peripheral blood of rabbits is best demonstrated by concentrating the white cells in a leukocytic layer by the centrifugation of a mixture of 3 cc. of blood and 2 cc. of 3.8 per cent sodium citrate. After removal of the citrated plasma, 6 drops of the buffy coat is transferred to 5 cc. of a dilute neutral red solution made by adding 1 part of saline solution saturated with neutral red (Gruebler's) to 19 parts of saline solution. After 10 minutes the mixture is centrifugated, and all the supernatant liquid removed except a volume equal to that of the cells. With platinum loop the cells are placed at the center of a cover-glass rimmed with vaselin which is at once inverted on a slide. The lymph-endotheliocytes with the neutral red in the hof of the nucleus number about 4 per cent. By the intraperitoneal injection of 10 cc. or more of whole rabbit's blood on three or four successive days the number is increased to 6 or 7 per cent.

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[5] Sabin, F. R., Doan, C. A., and Cunningham, R. S., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 330.

[6] McJunkin, F. A., *Am. J. Path.*, 1925, i.

Hem-endotheliocytes have not been demonstrated in the peripheral blood of normal rabbits. On the intravenous injection of 4 or 5 doses of 5 cc. of India ink at about 5-day intervals, there appears in the peripheral blood, within 24 hours after an injection, a cell identical with the hyaline type observed in the peritoneal exudates. This hem-endotheliocyte is characterized by one or more carbon particles in a clear cytoplasm practically devoid of neutral red. A maximum of 4 per cent has been observed. The appearance and disappearance of these leukocytes is subject to much variation. Three cc. of blood is readily obtained by puncture of marginal ear vein (ear other than the one used for ink injections) of the animal placed head downward in a box. Films on slides may be fixed for 2 hours in a solution consisting of 5 parts of commercial formalin and 95 parts of Zenker's fluid without acetic acid. The fixed films are transferred to 10 per cent formalin for 5 minutes, stained with hematoxylin (Harris without acetic) for 2 minutes, washed for a few seconds, and blotted dry at once. The neutral red is preserved in the cells that react to it.

2829

The effect of surface active substances on the diffusion of water through membranes.

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According to the investigations of Jacques Loeb,[1] the diffusion of water through membranes is a linear function of the solute, as would be expected according to the law of van't Hoff, only if the concentration of the salt solution is greater than M/16. In lesser concentrations than this the so-called "initial rate" of diffusion is represented by a curve having a maximum corresponding to a concentration varying from M/512 to M/256. If the concentration becomes greater the rate of diffusion decreases until a concentration of M/16 is reached. The experiments of Loeb, and of Bethe and Toropoff[2] seem to indicate the identity of electro-osmosis and free osmosis in low concentrations, and in this way furnish the explanation of the influence of electrolytes on the rate of diffusion by the difference of effect of the ions on the quantity of charge on the Helmholtz double layer on the capillary walls. Grumbach[3] has shown that surface active substances exert an influence on capillary electric forces and consequently on the diffusion potential.

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[1] Loeb, Jacques, *J. Gen. Physiol.*, 1919, ii, 189.

Our own investigations are concerned with the influence of surface active substances on the capillary kinetic phenomena.

Collodion sacs of 50 cc. capacity were attached to upright manometer tubes of 2 mm. diameter. The sacs were made on the inside of a 50 cc. Erlenmeyer flask, coated twice with 4 per cent collodion, and dried for one-half hour after the first coating, and 4 to 5 hours after the second. The sacs were filled with electrolytes of M/32 concentration immersed in distilled water.

In the case of a given electrolyte the manometer readings obtained with different sacs were relatively constant. Having established the normal rate of diffusion, the sacs were treated by filling them with solutions of surface active substances. After five to fifteen minutes the sacs were thoroughly rinsed, and again filled with electrolyte solutions. It was noted that this treatment of the membrane resulted in a very different rate of diffusion, as measured by the change of height of water in the manometer.

In the case of sodium chloride, sodium sulphate and sodium citrate and potassium ferrocyanide, the diffusion of water through the membranes into the electrolyte solutions was increased. The surface active substances causing this phenomenon were caproic acid, methylamine, ethylamine, theobromin sodiosalicylate, sodium oleate and sodium glycocholate, the latter two substances producing the greatest alteration in the membrane.

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[2] Bethe, A., and Toropoff, T. H., *Ztschr. f. Physiol. Chem.*, 1914, lxxxviii. 656; lxxxix, 597.

[3] Grumbach, *Ann. d. Chim. et de Phys.*, (8) 24,433, (1911); *Journ. d. phys.*, 2,283,385, (1912). Quoted by Freundlich, Kapillarchemie, Leipzig, 1922, 358.

In the case of certain electrolytes with polyvalent kations, the rate of positive osmosis was decreased. In the case of calcium chloride solution in sacs previously treated with sodium glycocholate, actual negative osmosis occurred, *i. e.,* water diffused from the electrolyte solutions into distilled water. This negative osmosis cannot be explained on the basis of valency alone, as aluminium salts fail to exhibit the phenomenon. The negative osmosis increases with the degree of dilution up to a maximum of M/64. At a concentration of M/8 the negative osmosis is completely compensated by the normal osmotic pressure. Repeated and long continued washing of the membranes after treatment with the surface active substance tended to decrease the negative osmotic effect. On the other hand, it was found that the phenomenon was obscured if an appreciable amount of surface active solution was allowed to remain in the sac.

Negative osmosis has been described by other authors in the case of certain acids, but not in the presence of neutral salts.[1] In Table I are given protocols of a typical experiment.

TABLE I.

Electrolyte solutions in sac.	Manometer Readings.					
	Before treatment of membrane.		After treatment with sodium glycocholate (M/40).		After treatment with sodium oleate (M/10).	
	Minutes	mm.	Minutes	mm.	Minutes	mm.
Na_2SO_4 (M/32)	20	+37	—	—	10 20	+60 +82
Na_2SO_4 (M/32)	20	+68	5 10	+62 +124		
Sod. Citrate (M/32)	20	+60	4	+90		
Pot. Ferrocyanide (M/32)	20	+78	5	+172		
Pot. Ferrocyanide (M/32)	20	+75			20	+132
$CaCl_2$ (M/32)	20	+7			20	balance
$CaCl_2$ (M/32)	20	+4	20	—19		
$CaCl_2$ (M/64)	20	+6	20	—39		
$CaCl_2$ (M/128)	20	+5	20	—16		

2830

Trypsin and insulin injections into the pancreatico-duodenal artery.

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According to Epstein[1] and co-workers, injection of trypsin, insulin, or saline solutions into the pancreatico-duodenal artery results in a hyperglycæmia and glycosuria in cats. Injection of trypsin into the portal vein also causes hyperglycemia, but NaCl or insulin does not lead to hyperglycæmia. On the basis of these results, certain conclusions were reached concerning the nature of the diabetic condition.

We have repeated these experiments on dogs, under conditions similar to those of Epstein and co-workers, with negative results. In no instance did glycosuria occur, nor were any marked increases of the blood sugar produced by the injection of solutions of trypsin, insulin or sodium chloride into the pancreatico-duodenal artery of dogs under amytal anesthesia. Following the injection of insulin into the pancreatic artery, the typical fall of blood sugar was regularly observed.

Dog No. 1. Injection of insulin into the pancreatico-duodenal artery.

Young female dog, weight 6.4 kg. 60 mg. amytal (Lilly) per kg. intraperitoneally. Catheterized. Laparotomy 20 minutes later; 3 cc. of insulin solution (12 units) injected into the pancreatico-duodenal artery. Abdomen closed in two layers.

Time	Blood sugar Shaffer-Hartmann Method	
	Per cent	
11:30 a. m.	.111	
11:40		Injection of 3 cc. (12 clinical units) into the artery.
11:41	.111	
11:48	.127	
12:01 p. m.	.083	
12:40	.056	
1:35	.032	
3:30	.100	Urine at no time was positive when tested with the Shaffer-Hartmann sugar reagent.

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[1] Epstein, Rosenthal, et al., *Am. J. Physiol.*, 1925, lxxi, 316.

Dog No. 2. Injection of saline and trypsin into pancreatico-duodenal artery.

Young female dog, weight 5.6 kg. 60 mg. per kg. amytal intraperitoneally. Abdomen closed in three layers.

3 cc. normal saline at 9:22 a. m. March 7.

Time	Blood sugar Shaffer-Hartmann Method	Remarks.
9:08 a. m.	.104	Amytal at 8:30 a. m.
9:23	.106	Laparotomy at 9:15 a. m.
9:27	.101	Abdomen closed immediately after injection.
9:30	.102	
10:17	.094	
10:39	.097	
10:57	.101	
11:26	.101	
1:45 p. m.	.089	
5:00	.103	Urine sugar 0 at all times.

March 8. Dog in good condition; eating meat.

10 cc. very active trypsin in saline at 12:15 p. m. March 13.

Time	Blood sugar Shaffer-Hartmann Method.	Myers-Benedict Method.
11:14 a. m.		.095
11:22	.090	.104
12:15 p. m.	(10 cc. trypsin into pancreatico-duodenal artery.)	
12:20	.101	.113
12:26	.97	.108
12:36	.101	.108
1:00	.098	.106
2:27	.092	.094

Tests of all urine specimens for sugar were negative.

Dog No. 3. Injection of normal saline and trypsin into the pancreatico-duodenal artery.

Young female dog, 4.8 kg. 60 mg. amytal per kg. intraperitoneally. Catheterized

4 cc. saline at 9:54 a. m. March 6.

Time	Glucose Shaffer-Hartmann Method.	Remarks.
9:33 a.m	.112	
9:54	Injection 4 cc. saline	
10:15	.118	
10:34	.123	
10:55	.110	
11:20	.127	
12:45 p. m.	.106	
5:00	.092	Urine sugar 0 at all times.

March 7. Animal eating meat; in good condition.

11 cc. Trypsin* in saline at 11:33 a. m. March 13.

Time	Blood Sugar	
	Shaffer-Hartmann Method	Myers-Benedict Method
11:00 a. m.	.122	.119
11:33	Injection 11 cc. trypsin	
11:18	.125	.114
11:25	.122	.110
11:36	.106	.114
11:47	.106	.114
12:00	.103	.107
12:34 p. m	.114	.109
1:04	.102	.107
2:32		.114

Urine sugar 0 at all times.
Dog killed at 2:45.

*Aqueous solution purified as suggested by Epstein and Rosenthal.[1]

Dog No. 4. Injection of trypsin into the pancreatico-duodenal artery.

Female animal, wt. 5.75 kg. 60 mg. amytal per kilo intraperitoneally.
11 cc. trypsin solution injected at 3:41 p. m.

Time	Blood Sugar		
	Shaffer-Hartmann Method	Epstein Method	
3:14	.101	.075	
3:21	.104	.091	
3:41			Injection of trypsin
3:43	.121	.132	
3:49	.113	.150	
4:00	.101	.200	
4:20	.111	.125	
4:39	.117	.150	
5:40	.114	.155	
9:50	.122	.138	

Urine negative for sugar at all times.

Dog No. 5. Injection of trypsin into the portal vein.

Male animal, wt. 9.4 kg. 60 mg. per kilo, amytal injected intraperitoneally.
13 cc. trypsin solution injected into portal vein.

Time	Blood Sugar			
	Shaffer-Hartmann Method	Myers-Benedict Method	Epstein Method	
4:45	.113	.105	.126	
4:54	.106	.099	.116	
5:00				Injection of trypsin
5:03	.108	.103	.113	
5:08	.114	.097	.113	
5:18	.103	.104	.116	
5:40	.100	.100	.132	
6:00	.100	.099		
6:30	.102	.102	.117	
7:00	.102	.101		
9:05	.101	.093	.129	

Urine negative for sugar at all times.

Dog No. 6. Injection of trypsin into the portal vein.

Female dog. Wt. 9.8 kg. 60 mg. amytal per kg. intraperitoneally.
8 cc. trypsin injected into portal vein.

Time	Blood Sugar		
	Shaffer-Hartmann Method	Epstein Method	
(1) 1:07	.109	.126	
(2) 1:24	.106	.116	
1:32			Injection of trypsin
(3) 1:33	.114	.113	
(4) 1:41	.113	.113	
(5) 1:48	.101	.116	
(6) 1:55	.103	.132	
(7) 2:22	.108		
(8) 3:00	.100	.117	
(9) 4:05	.093		
(10) 5:55	.093	.129	

Urine negative for sugar at all times.

2831

Observation on the carbohydrate metabolism of acetone-butyl alcohol fermentations.

GEORGE W. FREIBERG (Introduced by P. A. Shaffer).

[*From the Laboratory of Biological Chemistry, Washington University, School of Medicine, St. Louis, Mo.*]

A study of the general reactions involved during acetone-butyl alcohol fermentations was undertaken. A general carbon balance was established in which 95 to 98 per cent of the carbon was accounted for. Efforts to write general reactions were not successful until different stages of the metabolism were analyzed separately. During the true metabolism stage, during which most of the acetone and butyl alcohol is formed, it was found that acetone is produced in accordance with the general reaction

$$C_6H_{12}O_6 + H_2O \rightarrow C_3H_6O + 3\ CO_2 + 4\ H_2;$$

while the butyl alcohol was formed as follows:

$$C_6H_{12}O_6 \rightarrow C_4H_{10}O + 2\ CO_2 + H_2O.$$

Additional products, not accounted for by a disappearance of carbohydrate, arose as a result of the reduction of acetic and butyric acids to the corresponding alcohols, and involving a decrease in the amount of H_2 evolved with a corresponding increase in the percentage of CO_2. The increase in the percentage of CO_2 is only "apparent" and not actual as far as the real carbohydrate metabolism is concerned.

During the initial growth of the culture, acetic and butyric acids are produced. In analyzing this phase one encounters the difficulty that a certain amount of carbohydrate is incorporated into the protoplasmic structure of the cells. Further study on this phase is necessary, but it is thought that the general reactions involved are of a similar character to those of the metabolism phase. Acetic acid may possibly be produced as follows:

$$C_6H_{12}O_6 + H_2O \rightarrow 2\ CH_3COOH + 3\ H_2 + CO_2 + (\text{C and O, which are incorporated into the cell structure}).$$

Similarly

$$C_6H_{12}O_6 \rightarrow C_4H_8O_2 + CO_2 + 2\ H_2O + (\text{C, used in building up cell tissue}).$$

On the other hand an intramolecular rearrangement in accordance with the metabolic requirements and activities of the cells would yield only the neutral products. The possibility of glucose being broken up as follows:

$$C_6H_{12}O_6 \rightarrow C_4H_8O_2 + 2\ H_2 \text{ and } C_6H_{12}O_6 \rightarrow 3\ CH_3COOH,$$

and the possibility that all these reactions may take place simultaneously is not excluded as far as experimental evidence now at hand is concerned. With respect to the utilization of glucose by butyric acid forming organisms the literature presents some equations which involve hundreds of molecules. One is hesitant about accepting such reactions both because of the huge proportions and also because they appear to serve primarily to give a balanced written equation without taking into account the nutritive requirements of the organism.

2832

The tolerance of normal and phlorhizinized dogs for aceto-acetic acid.

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(Introduced by P. A. Shaffer).

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Intravenous injections of neutral freshly aerated sodium acetoacetate solutions, injected into dogs at a constant rate and over a long period of time, showed that the tolerance for acetoacetate is very great. Acetoacetate is almost completely tolerated (*i. e.,* disappears) in normal dogs when injected at a rate up to 5 or 6 millimols per kilo body weight per hour, the amount disposed of (or tolerated) being roughly proportional to the amount injected. A small portion of that injected is excreted as B-oxybutyric acid and acetoacetic acid in the urine, in the ration of from 1:1 to 2:1, and as acetone in exhaled air. Long continued phlorhizination and starvation does not abolish the tolerance, but does decrease it about 30 to 50 per cent below the normal. Injection of insulin into a phlorhizinized dog caused an immediate increase of tolerance which became normal in about three hours.

2833

The photochemistry of cod liver oil.

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Summary. The results of Kugelmass and McQuarrie reported in *Science,* Vol. 60, No. 1551, Sept. 19, 1924, in which cod liver oil and other antirachitic substances were found to radiate ultra-violet light upon oxidation with atmospheric oxygen in alkaline solution have not been confirmed. The positive results obtained by the authors were traced to faulty technique and presumably were due to black body radiation, since controls gave the same photographic results as the cod liver oil.

2834

The ether tension of surgical anesthesia.

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Recent work by Haggard[1] and by Shaffer and Ronzoni[2] has confirmed the idea, first advanced by Snow in 1848 but since often overlooked, that the absorption of ether in the lungs is a simple diffusion, and that the concentration of ether in the blood is therefore a function of the tension of ether in the lung air. The distribution ratio of ether between blood and air at 38° C. is about 15, according to which the tensions in air in equilibrium with the blood concentration required to produce anesthesia in dogs (110 to 130 mg. per cent) (Nicloux) are 19 to 23 mm. (2.5 to 3.0 vols. per cent) values which are much below the tensions commonly used with the Connell machine for the anesthesia of human patients. The concentration used by Boothby[3] for example, 51 mm. tension, if inhaled without dilution until the blood attains equilibrium would correspond to 293 mg. per cent in the blood, an amount which considerably exceeds the amount which causes respiratory failure (Nicloux, Ronzoni). The apparent discrepancy is due to the fact that the air inhaled by the subject is a mixture of that from the machine and of atmospheric air; the actual anesthetic tension in the air breathed is much below that delivered by the machine. White[4] has reported data on human patients which agree well with the data on dogs.

The present communication reports further data on the direct determination of ether in the blood of patients during surgical ether anesthesia. The results confirm the figures of the earlier workers on dogs, and indicate very similar range of anesthetic concentrations in human subjects.

For deep anesthesia a range of 110 to 170 mg. per 100 cc.

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[1] Haggard, H. W., *J. Biol. Chem.*, 1923, lv, 131.

[2] Shaffer, P. A., and Ronzoni, E., *J. Biol. Chem.*, 1923, lvii, 741; Ronzoni, E., *J. Biol. Chem.*, 1923, lvii, 761.

[3] Boothby, W. M., *J. Pharmacol. and Exp. Therap.*, 1913-14, v, 379.

[4] White, J. C., *Archives of Surgery*, 1923, vii, 347.

was found; for light anesthesia 41 to 110 mg. per 100 cc. of blood. These patients had all had morphia 1/6 gr. and atropine 1/150 gr. preliminary to operation. Another group of patients who had received hyoscine as well as morphine before operation were sufficiently relaxed by concentrations of 39 to 100 mg. of ether per 100 cc. so that abdominal operations were satisfactory.

A few direct determinations of the ether in the alveolar air of patients gave results below 25 mm. tension.

2835

Quantitative experimental evidence on the acuity for hearing by bone transmission.

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The type of apparatus employed in this series of observations has been previously described in this journal.[1] We submit some very general conclusions based on the observations made on individuals with known sensitivity for air-transmitted sound. A continuous tone scale was employed and the threshold approached from below as in the air tests.

Sensitivity for tone-transmission of sound varies a great deal more than that for air-transmitted sound, and is not definitely related to it. The sensitivity at any given frequency is not a criterion of the sensitivities at other pitches. We found about 500 v. p. s. the least variable. Areas of marked decrease in air acuity are similarly registered through the bone.

In normal ears the hearing is most acute at normal pressure for both air and bone transmitted sound. The development of plus and minus pressures in the air of the external auditory canal decreases acuity for both series of tests, and leads to the conclusion that the cranio-tympanic conception of sound transmission is probably the correct one.

In comparing the acuity for bone-transmitted sound, with external canals open, as opposed to external canals occluded, some rather disconcerting findings were made. The individual with the greatest sensitivity through the bone with the ears open showed the least advantage on closing the external canals. It was also observed that the enhancement in bone transmission due to occlusion of canals becomes less pronounced as the pitch rises, and is most marked in the low part of the tone scale.

2836

Quantitative experimental data on the rôle of the sympathetic innervation in the tonus of the quadriceps femoris muscles.

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By the use of Spiegel's method of measuring the tonus of the *quadriceps femoris* in resting animals,[1] tonus curves of this muscle in cats and dogs were obtained before and after elimination of its sympathetic nerve supply, and before and after removal of the cerebellum. In some of the experiments tonus curves of the same muscle were obtained before and after extirpation of the sympathetic trunk in the lumbar region, in others tonus curves of both quadriceps muscles were obtained in animals which had previously been subjected to unilateral extirpation of the lumbar sympathetic trunk. Removal of the cerebellum was commonly carried out after the tonus curves of both quadriceps muscles were obtained in animals which had been previously subjected to unilateral extirpation of the lumbar sympathetic trunk. Then the tonus curves of both muscles were obtained once more.

These tonus curves are essentially the curves of passive extension of the quadriceps muscles. Those obtained for the same muscle before and after elimination of its sympathetic nerve supply are practically identical with the curves obtained for the muscle on the unoperated and operated sides respectively, following unilateral extirpation of the lumbar sympathetic trunk. The time interval between sympathectomy and the measurement of tonus is relatively unimportant.

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[1] Spiegel, E. A., *Ztschr. f. d. ges. Neur. u. Psych.*, 1923, lxxii, 517.

Curves which may be regarded as typical of a large number obtained in these experiments are illustrated in the accompanying figure (Fig. 1). The curve of extension of the normal quadriceps (R) rises very slowly at first, and then more rapidly as the angle of flexion of the knee increases. The quadriceps muscle offers greater resistance at the beginning of passive displacement of the leg from a position of rest in which the knee is only slightly flexed than it does from one in which the angle of flexion of the knee is relatively large. This is known as the "brake phenomenon." The curve of extension of the *quadriceps femoris* deprived of its sympathetic nerve supply (L) rises rapidly from the beginning. This indicates less tonus than is exhibited by the normal muscle, and absence of the "brake phenomenon." Following removal of the cerebellum, the curve of extension of the quadriceps with its sympathetic nerve supply intact (R′) falls between

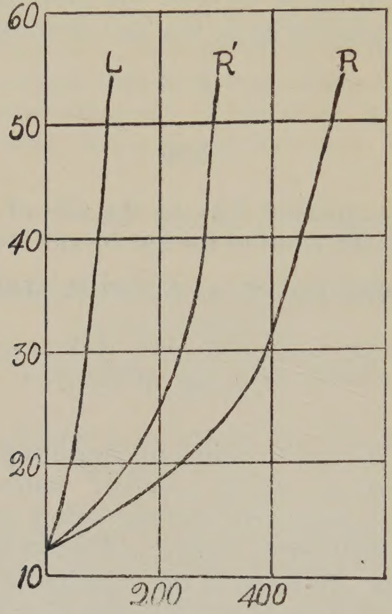

FIGURE 1.

Tonus curves of right and left quadriceps muscles of a cat obtained fourteen days after extirpation of the left lumbar sympathetic trunk. R, curve of normal right quadriceps; L, curve of left quadriceps; R′, curve of right quadriceps following removal of the cerebellum. The figures in the vertical series indicate the angle of flexion of the knee; those in the horizontal series indicate the force applied.

the two curves previously obtained, while the curve of extension of the quadriceps deprived of its sympathetic innervation coincides with the one obtained for this muscle when the cerebellum was intact. Obviously, the "brake phenomenon" depends in part on cerebellar impulses which are conveyed to the skeletal muscles through the sympathetic nervous system.

The component of muscle tonus which, as indicated by the curves obtained in these experiments, is mediated through the sympathetic nervous system, includes those elements of tonus which enable the muscle to develop resistance to passive displacement of a limb from a position of rest. These are the elements of tonus which tend to maintain any given posture, once that posture is imposed by voluntary effort or external force. They are closely akin to, perhaps identical with, those elements of tonus on which the "shortening" and "lengthening" reactions depend which Sherrington described and interpreted as the manifestations of plastic tonus.[2] Therefore, the results of these experiments afford quantitative evidence which supports the theory advanced by Langelaan[3] and more recently advocated by Hunter[4] and Royle[5] that plastic tonus in skeletal muscles is mediated through the sympathetic nervous system.

2837

The effect of the administration of thyroxin upon the surface tension of blood.

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In a previous communication[1] we have shown that thyroidectomy brings about in the course of nineteen to twenty-two days an increase in the value of the surface tension of the blood of guinea pigs. Since hypothyroidism causes this increase in the surface tension, it was of course of interest to ascertain whether the presence of excessive quantities of thyroid secretion or hormone would have the reverse effect upon the surface tension of the blood.

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[2] Sherrington, C. S., *Proc. Roy. Soc.*, 1896, lx.

[3] Langelaan, J. W., *Brain*, 1915, xxxviii, 235.

[4] Hunter, J. I., *Brain*, 1924, xlvii, 261.

[5] Royle, N. D., *Brain*, 1924, xlvii, 275.

[1] Wilhelmj and Fisher, PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 478.

We therefore fed thyroxin to normal guinea pigs. The feeding was continued over a period of three to four days, and from 1 to 1.2 milligrams were fed to each animal. The animals were bled before feeding, and again on the fourth or fifth day after beginning the feedings. Determinations of the surface tension of the blood plasma were made on both of these samples. At the same times normal animals were bled and, in order to have controls of unfed animals, they were kept under the same conditions as the ones used in the experiments.

Twenty-seven animals were fed thyroxin; of these, twenty showed a fall in the value of the surface tension of the blood which was greater than 1 dyne. The remaining seven animals showed a change of less than 1 dyne, some even showing a slight increase of the surface tension. The fall in the surface tension in the twenty animals varied from 1.9 dynes to 13.9 dynes. The average fall of surface tension between the first and second samples in all twenty-seven animals was 3.7 dynes; if we consider only those which showed a fall greater than 1 dyne, the average for these twenty is 5 dynes.

Of the seven control animals kept under the same conditions as the fed animals, six showed variations between the first and second determinations of less than 1 dyne, showing either a rise or a fall; one animal, however, showed a fall of 1 dyne. The average change in the surface tension of the control animals was a fall of .15 dynes.

We have added thyroxin directly to freshly drawn plasma in order to determine whether it was increased thyroxin content in the blood which might account for the lowered surface tension in the fed animals, but were unable to show that thyroxin had any surface active qualities.

It appears, then, from our experiments, that hyperthroidism brought about by administration of thyroxin causes a lowering of surface tension of plasma in guinea pigs, acting therefore in the opposite direction from hypothyroidism brought about by thyroidectomy. Furthermore, this lowering of the surface tension of the plasma is apparently not due to the presence in the blood of increased quantities of thyroxin.